JOURNAL OF AGRICULTURAL RESEARCH

VOL. 50 WASHINGTON, D.C., FEBRUARY 15, 1935 NO. 4

FACTORS AFFECTING INFECTION AND DECAY OF SWEETPOTATOES BY CERTAIN STORAGE ROT FUNGI [1]

By J. I. LAURITZEN

Senior physiologist, Division of Sugar Plant Investigations, Bureau of Plant Industry, United States Department of Agriculture [2]

INTRODUCTION

Rhizopus soft rot (*Rhizopus nigricans* Ehr. and *R. tritici* Saito), surface rot (*Fusarium oxysporum* Schl.), Java black rot (*Diplodia tubericola* (Ell. and Ev.) Taub.), charcoal rot (*Sclerotium bataticola* Taub.), and fusarium end rot, caused by species of *Fusarium* (including *F. oxysporum*), are the principal storage diseases of sweetpotatoes (*Ipomoea batatas* (L.) Lam.), and they have received major consideration in this paper.[3] The factors affecting the invasion of the tissue of sweetpotatoes during storage by fungi other than those listed above have also received some attention.

The object of the present investigation was to study the effect of the following factors on infection and decay of sweetpotatoes by the storage rot fungi just mentioned: Presence or absence of the pathogene, infection and decay by other fungi, amount of inoculum, wounding, suberization, periderm formation, temperature, humidity, and time. These factors will be considered in connection with the various diseases. A study was made also of the effect of temperature on the growth of *Diplodia tubericola* and *Sclerotium bataticola* in culture media. The investigations were made in Washington, D. C., and at the Arlington Experiment Farm, Rosslyn, Va.

REVIEW OF LITERATURE

Rhizopus tritici and *R. nigricans* are unable to attack or penetrate (*5, 11*) the unbroken skin of the sweetpotato. When roots are wounded these fungi are able to dissolve the middle lamellae (*3, 4, 5*) of the cells beneath the skin, thus separating the cells and disintegrating the tissues. Hauman-Merck (*7*) found that *R. nigricans* (*Mucor stolonifer* Ehr.) was unable to pass through cork formed in wounded sweetpotato tissue. Weimer and Harter (*13*) showed that a wound periderm was an effective barrier against infection of sweetpotatoes by *R. tritici*. They observed periderm formation over a range of temperature from 19.5° to 33° C. at relative humidities ranging from 95 to 97 percent. A periderm was not formed at a low humidity (percentage of relative humidity not given). They also observed that suberization preceded periderm formation.

[1] Received for publication Oct. 12, 1934; issued April 1935.

[2] Formerly pathologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry.

[3] Although black rot (*Ceratostomella fimbriata* (Ell. and Hals.) Elliott) is a serious storage trouble, it is also a field disease of some importance and in this respect is distinguished from the foregoing storage diseases. Some of the factors affecting its initiation and development in storage have been considered in a previous publication (*8*).[4]

[4] Reference is made by number (italic) to Literature Cited, p. 329.

A definite relation has been found between infection by *Rhizopus* (*12*) (which probably included both *R. tritici* and *R. nigricans*) and relative humidity at a temperature of 23° C.

Artschwager and Starrett (*1*)[5] made an extensive study of suberization and periderm formation as influenced by temperature, air humidity, and time. Their work was an outgrowth of the present investigation and was run under the same conditions and in conjunction with some of the infection experiments. The range of temperature at which suberization and periderm formation took place extended from 12.5° to 34.8° C. This lower temperature limit for cork formation is 7° below that obtained by Weimer and Harter (*13*), who, however, did not employ temperatures between 11.2° and 19.5°. No effort was made to determine the upper limit of periderm formation. In the Artschwager-Starrett experiments periderm formation took place in 3 to 5 days at temperatures above 18° (combined with high humidity), at 18° in 6 days (not included in published data), at 14.7° in 11 days, and at 12.5° only after 25 days. Suberization was found to precede periderm formation, and suberization as well as periderm formation was found to be related to the percentage of humidity at which the roots were stored. At a temperature of 12.5°, suberization and periderm formation were not obtained at a relative humidity below 93 to 94 percent. At 23°, suberization was not obtained below 76 percent relative humidity nor periderm formation below 87 percent within the limit of 10 days. At 28°, suberization occurred at relative humidities ranging from 66 to 95 percent; periderm, however, was not formed within 11 days at 66 percent relative humidity. It will be seen from these data that the higher the temperature the wider was the range of humidities at which both suberization and periderm formation took place.

Wounding and curing (*9*) have been shown to be important factors affecting infection of sweetpotatoes by surface rot (*Fusarium oxysporum*).

It has been suspected at times that invasion and possibly infection by certain storage-rot organisms may have been secondary to infection and decay by other fungi. Forms of *Fusarium* sometimes invade (*2*) mottle-necrosis lesions caused by species of *Pythium*. Forms of *Fusarium* also (*10*) invade lesions produced by such organisms as *Mucor racemosus* Fres., *Botrytis cinerea* Pers., and *Alternaria* sp. at temperatures ranging from 0° to 5° C., when the infected roots are stored at storage-house temperatures (10° to 15°).

At the inception of the present investigations, little information was available regarding the factors influencing the infection of sweetpotatoes by *Diplodia tubericola* and *Sclerotium bataticola*. *D. tubericola* (*6*) had been observed to infect sweetpotatoes through wounds, more readily in an open than in a closed vessel, to cause infection at temperatures of 12.2° to 13.5° and 34° to 35° C. more readily at the higher than at the lower temperature, and to completely decay roots in 4 to 6 weeks. *S. bataticola* (*6*) had been observed to infect sweetpotatoes through wounds and to completely decay roots in 3 to 6 weeks.

[5] To rectify any misconception that may have arisen as a result of statements in the introduction and in footnote 7 of the paper here cited (*1*), Artschwager has submitted the following: "The impression given by us, through the omission of a corrected statement, that our work consisted of more than a histological study of the materials furnished by J. I. Lauritzen is hereby corrected."

MATERIALS AND EQUIPMENT

The Yellow Jersey (Improved) (*9*) variety of sweetpotato was used in most of the experiments and the Porto Rico and Nancy Hall varieties were used in a few. The roots of all three varieties were grown and stored at the Arlington Experiment Farm, Rosslyn, Va.

In the experiments dealing with rhizopus soft rot, surface rot, and fusarium end rot, the sweetpotatoes were not inoculated and infection depended upon the pathogenes present on the roots. In the Java black rot and charcoal rot experiments, as well as in the temperature-growth experiments, *Diplodia tubericola* and *Sclerotium bataticola* isolated from sweetpotatoes were used.

The following storage space was employed in these experiments: Infection chambers 3 by 3 by 2 feet high (*11*), insulated rooms 8 feet in each dimension, and insulated cold-storage rooms 8 by 14 by 11 feet high.

The desired temperatures were obtained by heating (with electric heating coils) either the normal air or air cooled by refrigeration, and the temperature was controlled by electric thermoregulators. The relative humidity was controlled by the use of calcium chloride or water in evaporating pans, and by commercial humidifiers in the case of two of the large rooms when a high humidity was desired.

RHIZOPUS SOFT ROT

INFLUENCE OF TEMPERATURE AND HUMIDITY ON INFECTION

Influence of Humidity on Infection at 23° C.

In an earlier study (*12*) on the relation of humidity to infection of sweetpotatoes (Yellow Jersey) by *Rhizopus* at a temperature of 23° C., it was found that as the relative humidity fell from a range of 93 to 99 percent to a range of 76 to 84 percent the percentage of infection increased from a range of 10 to 21 to a range of 88 to 100 and decreased as the humidity continued to fall. At relative humidities ranging from 48 to 53 percent the percentage of infection ranged from 23 to 25 percent. It was found that a protective layer of tissue was formed over wounds in the uninfected roots exposed to the higher humidities that limited infection when these roots were stored at any of the humidities from 48 to 95 percent, while most of the uninfected roots from the lower humidities became infected when stored at humidities of 84 to 95 percent. In the suberization-periderm studies of Artschwager and Starrett (*1*), at a temperature of 23° relative humidities of 49, 65, 76, 87, and 95 percent were employed. The duration of storage at these humidities was 10 days. Suberization occurred at the 3 highest humidities only. A periderm was formed at the 2 higher humidities but not at the 3 lower. It will be seen, therefore, that periderm formation takes place within the range of humidities at which the infection barrier was developed in the Lauritzen-Harter experiments (*12*) and without doubt is the barrier involved. (The humidities employed in the suberization-periderm experiments (*1*) were too limited to determine exactly the range of humidities at which these processes occur.) Data confirming this conclusion were obtained in connection with the suberization-periderm experiment (table 1)[6] of the present investigation.

[6] The roots in this experiment were halved longitudinally in the same fashion as in the earlier experiment (*12*), and were stored without inoculation.

TABLE 1.—*Influence of humidity at a temperature of 23° C. on suberization and periderm formation and on the infection by Rhizopus of sweetpotatoes* [1] *during 10 days' storage*

[19 roots used at each humidity]

Temperature (° C.)	Relative humidity	Roots infected		Suberization [2]	Periderm formation [2]
	Percent	*Number*	*Percent*		
23.0	95	3	16	+	+
23.0	87	3	16	+	+
23.6	76	19	100	+	−
22.8	65	0	0	−	−
23.0	49	0	0	−	−

[1] The sweetpotatoes (Yellow Jersey Improved) were harvested Oct. 6 and 7, 1927, cured for 10 days at temperatures of 26° to 29° C., and stored at 10° to 15° and a relative humidity of 90 percent from Oct. 17 to Mar. 3, 1928.

[2] Plus or minus signs indicate presence or absence of suberization and periderm formation.

Although suberization occurred within 4 days at a relative humidity of 76 percent, it did not prevent infection. Infection was limited at relative humidities above and absent at relative humidities below 76 percent.

The fact (*12*) that uninfected halved roots first stored at relative humidities below 76 percent for a period became infected when stored for another period at relative humidities above 84 percent (23° C.) indicates that the limitation of infection during the initial storage was at least partly due to the effect of humidity on the pathogenes.

The foregoing data indicate: (1) That relative humidity above 76 percent at 23° C. is a factor favoring the development of suberization and periderm formation and limiting infection, (2) that suberization either is not an effective barrier in preventing infection at a relative humidity of 76 percent at 23° or it does not occur in time to exclude the initiation of decay, and (3) that a periderm is an effective barrier against infection under these conditions.

INFLUENCE OF HUMIDITY ON INFECTION AT 12° C.[7]

It was stated in an earlier report (*12*) that the results of one experiment, involving a study of the relation of humidity to infection of sweetpotatoes by *Rhizopus* at a temperature of 12° C., "correspond with those obtained at 23°, except that the range of infection was shifted nearer 100 percent humidity." In the light of the results of later and more comprehensive experiments this statement requires qualification, as the apparent resistance to infection in halved roots at high humidities at 12° is not as definite and permanent as at 23° and will yield greater infection during subsequent storage, especially at certain relative humidities.

Nine [8] experiments were conducted at different periods of the storage season during 3 years. The roots (Yellow Jersey Improved) were halved longitudinally with a sharp knife and stored at the various relative humidities (4 in each experiment) at 12° C. without inoculation. After preliminary storage of 9 to 11 days at the different relative humidities at 12° C. the roots were subjected to another period

[7] *Rhizopus nigricans* (*11*) is the pathogene that causes rhizopus soft rot at a temperature of 12° C.

[8] The roots used in 2 of the experiments were uncured; those used in the remaining experiments were cured for 10 days at a temperature ranging from 25° to 30° C.

of storage (11 to 34 days) at various humidities, at temperatures ranging from 12° to 15.5°. In 6 out of the 9 experiments the percentage of infection was less during the 10 days' preliminary storage at relative humidities ranging from 91 to 98 percent than at those ranging from 62 to 86 percent, with two exceptions (at a relative humidity of 62 percent in one experiment and 85 percent in the other). By the end of the subsequent storage period the percentage of infection was generally less in roots initially stored at relative humidities of 93 to 98 percent than in roots initially stored at the lower humidities. The percentage of infection was always less when the subsequent storage humidity was above 90 percent.

The association of an inhibition of infection of sweetpotatoes by *Rhizopus nigricans* with the formation of a suberin layer at relative humidities of 94 to 98 percent indicates that suberization is a factor limiting infection; however, it is not so effective a barrier as is a periderm. If periderm formation played a part in inhibiting infection at these humidities, it was only during the latter part of the storage period.

The failure of a suberin or periderm layer to form on wounded surfaces at the lower humidities explains the heavy infection in roots exposed under these conditions. If the relative humidity becomes low enough to limit infection, the inhibition is removed as soon as the roots exposed to such humidities are stored at the higher humidities (above 90 percent). This fact will become more evident from the results to be discussed presently.

In roots initially stored at a relative humidity of 93 to 97 percent at 12° C., followed by subsequent storage at 12° to 15° and various relative humidities, the percentage of infection was lower in the experiments started in the early part of the season (before Dec. 22) than in those started later. Earlier results (*11*) show that *Rhizopus* generally is less abundant on the roots early in the storage season than later and that freshly dug potatoes become infected as readily as old ones when they are inoculated by dipping in a water spore suspension.

Influence of High Humidities on Infection at 12° to 36.5° C. and of Different Humidities at 12°

Data were obtained from two experiments in which longitudinally halved, cured and uncured roots (Yellow Jersey) were initially and subsequently stored as indicated in table 2.

With the exception of 2 percent infection at 31° C. during the initial storage period, there was no infection by *Rhizopus* at any of the temperatures from 22° to 31° during either the initial or the subsequent storage periods. The results at the various humidities at 12° are similar to those already discussed. It should be noted that when storage was continuous at relative humidities of 96 and 97 percent the infection remained relatively small (5 and 17 percent) throughout the experiment, whereas in roots initially stored for 11 days at 96 to 97 percent relative humidity and subsequently for 20 days at relative humidities of 80 and 83 percent the percentage of infection was rather high (80 and 40 at 83 and 80 percent relative humidity, respectively). The percentage of infection, however, was not so high as in roots originally stored at relative humidities of 82 to 83, 73 to 80, and 60 to 67 percent, in which it reached 100 percent by the end of 10 days' final storage at 96 to 97 percent relative humidity.

TABLE 2.—*Influence of temperature and relative humidity on infection of sweetpotatoes by Rhizopus* [1]

Initial 11 days' storage						Subsequent storage [1]				
Storage conditions				Roots used (total for the 2 experiments) [1]	Roots infected (total for the 2 experiments) [1]	Storage conditions		Roots used	Total infection after—	
Experiment 1		Experiment 2								
Temperature (° C.)	Relative humidity	Temperature	Relative humidity			Temperature	Relative humidity		10 days	20 days
	Percent	*° C.*	*Percent*	*Number*	*Percent*	*° C.*	*Percent*	*Number* [2]	*Percent*	*Percent*
31	92	31	93	59	2	31	92	58	0	0
24	93	24	94	60	0	24	93	60	0	0
22.5	93	22	94	59	0	22.5	93	59	0	0
22	95	21.5	94	59	0	22	95	59	0	0
15.5	92	15.5	95	60	3	15.5	92	59	3	5
12	96	[3] 12	97	62	6	12	96–97	38	5	17
12	82	12	83	60	35	12	96–97	39	100	------
12	73	12	80	60	47	12	96–97	32	100	------
12	60	12	67	60	8	12	96–97	55	100	------
------	------	[3] 12	97	------	------	12	83	10	50	80
------	------	[3] 12	97	------	------	12	80	10	20	40

[1] With the exception indicated in footnote 3, the results given in this table are totals of 2 experiments. The sweetpotatoes were harvested Oct. 11, 1928. Experiment 1 was started immediately with uncured roots; experiment 2 was started Oct. 23, after 12 days' curing of the roots at 21° to 23° C.

[2] The numbers in this column at temperatures from 15.5° to 31° that do not correspond to the original numbers are instances in which the roots were removed from the experiment at the end of the preliminary storage period.

[3] Two lots of the roots originally stored at 12° C. and 97 percent relative humidity were stored subsequently at relative humidities of 80 and 83 percent, respectively.

There was some *Rhizopus* infection in roots stored continuously at a temperature of 15.5° C. It would seem that at this temperature there was a race between wound-periderm formation and infection. While Artschwager and Starrett (*1*) found periderm formation in one instance at 14.7° and 94 percent relative humidity in 11 days, it was not found in another case at 15.7° and 92 percent relative humidity in 10 days.

Another experiment was run (table 3) in which halved roots were first stored for a period of 10 days at a range of temperatures from 12° to 36.5° C. combined with high humidities at each temperature and a range of four humidities (from 68 to 96 percent) at 12°. After this period of initial storage, four lots of the uninfected roots from each of these combinations of temperature and humidity were stored, respectively, at relative humidities of 95, 82, 78, and 68 percent at 12° for ensuing periods of 10 and 20 days.

By the end of the preliminary storage period, 5 percent of the roots stored at 36.5° C. and a relative humidity of 96 percent and 2 percent of those stored at 22° and a relative humidity of 95 percent had become infected by *Rhizopus* (table 3). There was no infection between 22° and 36.5°, during either the initial or the subsequent storage periods. There was infection at 15.5° during the initial storage and by the end of 20 days of final storage at the three lowest humidities at 12°.

Some resistance to infection was built up in the roots stored originally at 12° C. and a relative humidity of 96 percent and it was maintained more strongly when the storage was continued at that temperature with approximately the same relative humidity than with lower humidities. All the roots originally stored at the 3 lowest humidities became infected after a period of further storage at 12°

TABLE 3.—*Influence of temperature and relative humidity on infection of cured sweetpotatoes by Rhizopus, Nov. 15 to Dec. 15, 1928*

Initial 10 days' storage				Subsequent storage															
				Infection in 10 days at 12° C. and relative humidity indicated								Infection in 20 days at 12° C. and relative humidity indicated							
				95 percent		82 percent		78 percent		68 percent		95 percent		82 percent		78 percent		68 percent	
Temperature	Relative humidity	Roots used	Roots infected	Roots used	Roots infected	Roots used	Roots infected	Roots used	Roots infected	Roots used	Roots infected	Roots used	Roots infected	Roots used	Roots infected	Roots used	Roots infected	Roots used	Roots infected
° C.	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*
36.5	96	60	5	15	0	14	0	14	0	14	0	15	0	14	0	14	0	14	0
31.5	90	60	0	15	0	15	0	15	0	15	0	15	0	15	0	15	0	15	0
24.5	94	60	0	15	0	15	0	15	0	15	0	15	0	15	0	15	0	15	0
22	95	60	2	15	0	15	0	14	0	15	0	15	0	15	0	14	0	15	0
22	93	60	0	15	0	15	0	15	0	15	0	15	0	15	0	15	0	15	0
15.5	95	60	3	15	0	15	0	14	7	14	0	15	0	15	27	14	7	14	43
12	96	60	15	13	23	13	69	13	46	12	8	13	38	13	85	13	69	12	58
12	81	60	87	2	100	2	50	2	100	2	50	------	------	2	50	------	------	2	50
12	78	60	63	6	100	6	33	5	40	5	20	------	------	6	50	5	40	5	20
12	68	60	0	15	100	15	7	15	40	15	0	------	------	15	53	15	73	15	47

and 95 percent relative humidity. Some of the roots from each of these 3 relative humidities remained uninfected when stored at a relative humidity of 68 percent, some of those originally stored at the 2 lowest humidities remained uninfected when stored at a relative humidity of 78 percent, and some of those originally stored at the 2 lowest humidities remained uninfected during the final storage at the 3 lowest humidities. More of the roots originally stored at 81 percent relative humidity became infected during the entire period of storage than roots from any of the other initial humidities. In this experiment temperature and humidity as they affect the host and humidity as it affects the pathogene become factors in the infection and decay of sweetpotatoes by *Rhizopus*. When temperature and humidity were such as to favor suberization and periderm formation (12° to 36.5° combined with high humidities), infection was limited because of the difficulty or inability of the pathogene to penetrate the suberized or periderm layers.

Infection at relative humidities of 68 and 78 percent at 12° C. was less during the initial storage period than at 81 percent relative humidity and remained less during subsequent storage periods at relative humidities of 68 and 78 percent. In the absence of suberization and periderm formation to inhibit infection at humidities of 68 and 78 percent, it would seem that it was the effect of humidity on the pathogene that limited infection during the initial storage, because when more moisture (at 95 percent relative humidity) was added to the environment the pathogene (*Rhizopus nigricans*) was able to initiate infection and decay.

Influence of Humidity on Infection at 28° to 29° C.

Seven experiments were run during three storage seasons (1928–29, 1929–30, and 1930–31) in which roots halved longitudinally were stored at 5 relative humidities in each experiment (except one in which there were only 4 relative humidities) at temperatures of 28° to 29° C. for a period of 10 days, and subsequently at temperatures from 10° to 16° at various relative humidities for another period of 19 to 31 days (table 4).

The results differed somewhat in the various experiments, for which reason they are submitted in detail. The factors that may have played a part in producing this variation were (1) the amount and uniformity of inoculum present, (2) the number of roots used, and (3) the variation of the subsequent storage.

The relation of relative humidity to infection was less definite in experiments run early in the season (experiments 2, 3, and 4) than in those run later, with the exception of experiment 5. In this latter case the number of roots used was too small to give an accurate picture of the effect of humidity on infection. The low relative humidity of the subsequent storage in experiments 4 and 5 may have played a part in producing the rather large percentage of infection in roots initially stored at a relative humidity of 96 percent. It has been observed that halved roots, healed over at high humidities, sometimes crack open in the wounded areas when they are subjected to storage at low humidities. Such wounds may give rise to new infections. If the results of the seven experiments are assembled and averaged in accordance with the initial storage humidities (table 5) it will be seen that there was a fairly definite relation between the rel-

ative humidity and the percentage of infection. Barring certain exceptions, the percentage of infection tended to increase with the lowering of the relative humidity. These results indicate that infection is inhibited over a fairly wide range of humidities at a temperature of 28° to 29° C. as a result of wound healing and that the percentage of infection is influenced by the rate of healing.

TABLE 4.—*Influence of relative humidity on infection of sweetpotatoes by Rhizopus at 28° to 29° C.*[1]

Experiment no.	Dates	Initial 10 days' storage				Subsequent storage			
		Temperature	Relative humidity	Roots used	Roots infected	Temperature	Relative humidity	Duration	Roots infected
		° C.	*Percent*	*Number*	*Percent*	*° C.*	*Percent*	*Days*	*Percent*
1	Feb. 12–Mar. 13, 1929	29	95	20	0	10	84	19	0
		29	91	21	0	10	84	19	0
		29	83	20	5	10	84	19	5
		29	74	18	0	10	84	19	11
		29	66	19	0	10	84	19	26
2	Oct. 11–Nov. 21, 1929	28	96	10	0	15.5	87	31	0
		28	90	10	0	15.5	87	31	0
		28	78	10	20	15.5	87	31	20
		28	69	10	0	15.5	87	31	0
		28	59	10	10	15.5	87	31	10
3	Oct. 11–Nov. 14, 1930	28	98	40	0	15.5	84	24	0
		28	84	40	2.5	15.5	84	24	55
		28	74	40	0	15.5	84	24	0
		28	72	40	0	15.5	84	24	40
4	Oct. 25–Dec. 5, 1929	29	96	10	0	15.5	58	31	50
		29	90	10	20	15.5	58	31	20
		29	87	10	0	15.5	58	31	0
		29	71	10	20	15.5	58	31	20
		29	63	10	0	15.5	58	31	0
5	Jan. 23–Feb. 24, 1930	29	96	6	33	13	50	22	50
		28	89	6	17	13	50	22	17
		28	78	6	17	13	50	22	17
		28	69	6	17	13	50	22	17
		28	62	6	17	13	50	22	17
6	Jan. 21–Feb. 21, 1931	29	96	80	2.5	15.5	92	21	2.5
		28	88	80	5.0	15.5	92	21	6.0
		28	78	80	34.0	15.5	92	21	34.0
		28	72	80	54.0	15.5	92	21	66.0
		29	62	80	66.0	15.5	92	21	75.0
7	Feb. 3–Mar. 5, 1931	28.5	96	80	36	16	92	20	36
		28.5	89	80	20	16	92	20	21
		28.0	77	80	48	16	92	20	50
		28.0	72	80	64	16	92	20	73
		29.0	62	80	53	16	92	20	60

[1] Uncured roots were used in experiments 2 and 3, and cured roots in the others.

TABLE 5.—*Influence of relative humidity on infection of sweetpotatoes by Rhizopus at 28° to 29° C., as based on data in table 4*

Relative humidity	Roots used	Roots infected			
		During initial storage		During initial and final storage	
Percent	*Number*	*Number*	*Percent*	*Number*	*Percent*
95–98	246	33	13	39	16
88–91	207	23	11	25	12
83–87	70	2	3	23	33
74–78	234	68	29	72	31
69–72	226	97	43	130	58
59–66	205	97	47	115	56

INFLUENCE OF TYPES OF WOUNDING ON INFECTION BY RHIZOPUS AT DIFFERENT TEMPERATURES AND HUMIDITIES

During the seasons 1928–29 and 1929–30, four experiments were conducted in which sweetpotatoes (Yellow Jersey) were subjected to 10 different types of wounding and stored without inoculation at 15 combinations of temperature and relative humidity (tables 6 and 7)

for a period of 10 days and then stored at a common temperature and relative humidity for another period. The temperatures and relative humidities of the subsequent storage period were as follows: Experiment 1, 10° C. and 84 percent relative humidity; experiment 2, 15.5° and 87 percent; experiment 3, 15.5° and 58 percent; and experiment 4, 13° and 67 percent. The duration of the subsequent storage was 19, 31, 31, and 21 days, respectively. The number of roots stored at each initial combination of temperature and humidity in each experiment was from 5 to 20, but in most cases 10. In three of the experiments the roots were cured and in one they were not.

TABLE 6.—*Influence of 10 types of wounding on infection of sweetpotatoes by Rhizopus at different temperatures and humidities during 10 days' storage* [1]

Type of wounding [2]	Percentage [3] of roots infected at indicated temperatures (°C., above) and relative humidities (percent, below)							
	35–37 91–95	31–32 88–95	28–29 95–96	25 91–94	21–22 91–94	19–21 89–93	15–16 89–95	12 93–98
Bruised tip	8.6	11.4	8.0	11.4	46.0	35.3	37.1	40.0
Bruised side	19.4	5.7	4.0	23.0	42.9	40.0	28.6	45.7
Broken tip	2.8	0	4.0	0	0	0	0	0
Cross section tip	0	0	0	0	0	2.9	0	0
Cross section medium	0	0	0	0	0	0	2.9	0
Cross section half	0	0	6.7	0	0	0	2.5	5.0
Longitudinal section shallow	2.9	0	0	0	2.9	0	0	0
Longitudinal section medium	0	0	0	0	0	0	0	0
Longitudinal section half	4.0	0	0	16.0	0	0	24.0	13.7
Skinned	0	0	0	0	0	0	0	0

Type of wounding [2]	Percentage [3] of roots infected at indicated temperatures (°C., above) and relative humidities (percent, below)								
	28–29 95–96	28–29 90–91	28–29 78–83	28–29 69–74	28–29 59–66	12 93–98	12 84–86	12 70–77	12 62–65
Bruised tip	8.0	0.0	0.0	0.0	0.0	40.0	5.7	0.0	2.9
Bruised side	4.0	8.3	24.0	0	4.0	45.7	51.4	45.7	20.0
Broken tip	4.0	0	4.0	0	0	0	0	0	0
Cross section tip	0	4.2	0	0	0	0	0	0	0
Cross section medium	0	0	0	0	0	0	5.7	0	0
Cross section half	6.7	0	0	5.3	0	5.0	20.0	15.0	2.5
Longitudinal section shallow	0	0	0	0	0	0	2.9	0	0
Longitudinal section medium	0	0	0	0	0	0	14.3	5.0	0
Longitudinal section half	0	4.9	7.5	5.1	2.6	13.7	24.0	34.0	26.5
Skinned	0	0	0	0	0	0	0	0	0

[1] The results given in this table were obtained from 4 experiments in each of which from 5 to 20 roots (10 in the majority of cases) were used for each type of wounding and for each combination of temperature and humidity. The experiments were started Dec. 22, 1928, and Feb. 12, Oct. 11, and Oct. 25, 1929. Cured roots were used in 3 experiments and uncured roots in 1.

[2] For definitions of types of wounding see text below.

[3] The percentages are based on from 35 to 50 roots.

A description of the various types of wounding follows. (1) One end of the roots was tapped on a laboratory desk, bruising the tissue from 5 to 10 mm deep (bruised tip). (2) The roots at their thickest diameter were struck on the rim of a wire basket, thus bruising the root tissue to a depth of about 5 to 8 mm (bruised side). (3) One tip end of the roots was broken off with the fingers at a point where the root was about 7 to 10 mm in diameter (broken tip). (4) One tip end was cut off with a sharp knife back from the tip where the root was about 7 to 10 mm in diameter (cross section tip). (5) The roots were cut in cross section about midway between the one tip end and the thickest diam-

eter of the root, and the larger portion was stored (cross section medium). (6) The roots were halved with a sharp knife in cross section (cross section half). (7) A small slice, about 1 to 1.5 cm in diameter, was cut from the side of the root between the skin and the outer vascular tissue (longitudinal section shallow). (8) A slice was taken from the side of the root to a depth of about one-fourth of its diameter (longitudinal section medium). (9) The roots were halved with a sharp knife longitudinally (longitudinal section half). (10) The roots were skinned in 2 to 3 places (the number of skinned areas on a root was the same in a given experiment) in areas from 8 to 10 mm in diameter (skinned).

TABLE 7.—*Influence of further storage at 10° to 15.5° C. and relative humidities of 58 to 87 percent on infection by Rhizopus of the roots discussed in connection with table 6* [1]

Type of wounding	Percentage [2] of roots infected as related to indicated initial temperatures (°C., above) and relative humidities (percent, below)							
	35–37 91–95	31–32 88–95	28–29 95–96	25 91–94	21–22 91–94	19–21 89–93	15–16 89–95	12 93–98
Bruised tip	8.6	11.4	8.0	11.4	46.0	35.3	40.0	45.7
Bruised side	19.4	5.7	4.0	23.0	42.9	40.0	28.6	45.7
Broken tip	8.3	0	4.0	0	0	0	8.6	2.9
Cross section tip	0	0	0	0	0	2.9	0	0
Cross section medium	0	0	0	0	0	0	5.7	8.6
Cross section half	2.5	0	10.0	0	0	0	5.0	20.0
Longitudinal section shallow	2.9	0	0	0	2.9	0	0	0
Longitudinal section medium	5.5	0	0	0	2.7	0	5.7	8.6
Longitudinal section half	6.0	0	12.5	18.0	2.0	2.0	46.0	56.9
Skinned	3.3	0	0	0	0	0	0	0

Type of wounding	Percentage [2] of roots infected as related to indicated initial temperatures (°C., above) and relative humidities (percent, below)								
	28–29 95–96	28–29 90–91	28–29 78–83	28–29 69–74	28–29 59–66	12 93–98	12 84–86	12 70–77	12 62–65
Bruised tip	8.0	8.3	0.0	0.0	0.0	45.7	14.3	0.0	2.9
Bruised side	4.0	8.3	24.0	0	4.0	45.7	54.3	54.3	40.0
Broken tip	4.0	0	4.0	0	0	2.9	8.6	0	2.9
Cross section tip	0	4.2	0	0	0	0	0	0	0
Cross section medium	0	0	0	0	4.0	8.6	25.7	34.3	28.9
Cross section half	10.0	0	0	9.4	6.7	20.0	47.5	67.5	47.5
Longitudinal section shallow	0	0	0	0	0	0	11.4	16.7	2.9
Longitudinal section medium	0	0	0	0	4.0	8.6	34.3	47.5	28.0
Longitudinal section half	12.5	4.9	7.5	10.3	15.4	56.9	72.0	98.0	84.0
Skinned	0	0	0	0	0	0	10.0	3.3	0

[1] The results given in this table were obtained from the 4 experiments discussed in connection with table 7. The duration of the storage subsequent to the initial 10 days' storage was 19, 31, 31, and 21 days, respectively. Values are total percentages of infection for initial and subsequent storage periods combined.
[2] Based on 35 to 50 roots.

The results from the four experiments on the presence or absence of infection were so similar that it was decided to combine them in order to simplify the presentation of the data. There was some variation in the percentage of infection under given conditions in a given experiment but not sufficient to alter the general relations found. Table 6 shows the percentages of roots infected in the four experiments at the end of the initial period of storage, and table 7 shows the percentages of infection for the initial and subsequent storage periods combined.

The data of tables 6 and 7 reveal marked differences in the percentage of infection as correlated with the type of wounding. During the

initial storage period at the various temperatures (combined with high humidities) infection was more consistently present in roots bruised at the tip and in those bruised at the side. By the end of the ensuing storage period the same was true in roots bruised at the tip, in those bruised at the side, and in those halved longitudinally. Infection was more uniformly present in roots with bruised tips and in roots with bruised sides than in roots halved longitudinally. Bruising interferes with healing (*13*) more than do the other types of wounding, and hence favors heavier infection. The higher percentage of infection in halved roots than in the other types of wounding, except bruising, is believed to be due to the greater area of wounded tissue. No infection by *Rhizopus* occurred on the skinned roots during the initial period of storage and very little during the ensuing period. There was either no infection or the percentage was relatively small on roots cut in cross section at the tip or broken tip either during the preliminary or subsequent storage. In general, the greater the extent of wounded area in cut roots the greater the percentage of infection. There was very little change in the percentage of infection following the initial storage in roots bruised at tip or side and originally stored at temperatures from 12° to 35° to 37° C. combined with high humidity. In the case of roots bruised at tip or side the percentage of infection was higher at temperatures ranging from 12° to 22° (combined with high humidity) than at temperatures above 22° at the end both of initial and of final storage. The initial storage at temperatures of 12° to 16° and high humidities generally yielded more infection (table 7) by the end of the final storage period in some of the types of wounding (cross section medium, cross section half, longitudinal section medium, and longitudinal section half) than did the initial storage at temperatures from 19° to 37°. Although generally there was little infection at any of the temperatures and humidities during either the initial or ensuing storage periods, in roots subjected to types of wounding designated as broken tip, cross-section tip, and longitudinal section shallow, it will be seen later that temperatures from 12° to 16° were critical in these roots as well as in roots subjected to the other types of wounding.

These results show that temperature combined with high humidity limited infection more in cut surfaces than in bruised tissue. In the former case cork formation was free to proceed at its normal rate, and above 15° to 16° C. this was sufficiently rapid to intercept most of the infection. At 16° and below, the rate of cork formation was retarded enough to permit of infection (in roots with the larger cut areas), which generally increased in percentage (tables 6 and 7) with the lowering of the temperature. The infection rate and the cork-formation rate curves cross somewhere between temperatures of 15° to 16° and 19° to 21°. In bruised tissue healing was inhibited and infection took place more or less readily at all the temperatures. The bruised tissue, in addition to interfering with healing, forms an excellent matrix for the pathogenes (*Rhizopus nigricans* and *R. tritici*) to establish a foothold in the host tissue. The fact that temperatures above 22° and at 28° to 29°, irrespective of humidity, tend to limit infection indicates the possibility that some suberization and periderm formation occur in bruised tissue at these temperatures. A periderm [9] has been observed to occur in bruised tissue at temperatures of 25° and 29°.

[9] Observations made by the writer and L. L. Harter in connection with a study of the effect of temperatures from 25° to 29° C. in checking decay of sweetpotatoes by *Rhizopus nigricans*.

In these experiments (tables 6 and 7) there was little relation between infection and relative humidity at 28° and 29° C., except possibly in roots halved longitudinally, by the end of the final storage period. Barring the rather high percentage (12.5 percent) of infection at relative humidities of 95 to 96 percent, there was an increase in the percentage of infection with lowering of the relative humidity. Whether the exception was accidental and associated with insufficient numbers to iron out individual variations or whether such high humidities at 28° to 29° are peculiarly favorable to the activities of the pathogenes is not known. Such exceptions have occurred in a number of experiments, but in others there has been no infection at all at this humidity. This tendency for the percentage to increase with the lowering of the relative humidity is correlated with the increase in time required for cork formation to take place with the lowering of the relative humidity. It would seem that the chances of infection are greater and those for complete healing less in longitudinally halved roots than in the other cut surfaces because of the greater wounded area of the former. The chances for complete healing might well decrease to a greater extent in the larger than in the smaller wounds with the lowering of the humidity. Infections would then be expected to increase with lowering of humidity provided the humidity of the ensuing storage did not too greatly inhibit the activities of the pathogenes. When the cut surfaces are limited, especially in cross section tip and cross section medium, and in longitudinal sections shallow and medium (tables 6 and 7), it appears that temperatures of 28° to 29° are sufficiently favorable to healing to more than counterbalance the retarding effect of an unfavorable humidity.

Artschwager and Starrett (*1*) obtained suberization at 28° C. at relative humidities of 73.5, 82.7, 90.6, and 95 percent within 48 hours and periderm formation at relative humidities of 90.6 and 95 percent within 96 hours, at 82.7 percent within 120 hours, and at 73.5 percent within 144 hours. At 66 percent relative humidity, suberization occurred in 6 days but no periderm was formed within 11 days. This humidity, as well as that of 73.5 percent, probably has some effect on the pathogenes inhibiting infection during the initial storage period, and since suberization occurred by the end of 6 days it is probable that its formation and a possible later formation of periderm may have limited infection during the subsequent storage period.

In roots stored at the various relative humidities at 12° C. the different types of wounding had a marked influence on the amount of infection. During the initial storage period rather heavy infection occurred in roots that had been bruised at tip, bruised at side, or halved in cross section or longitudinally. During the ensuing storage period the percentage of infection increased quite definitely with the extent of wounding in cut surfaces, no infection occurring in the cross-section-tip type and the percentage remaining comparatively small in the longitudinal-shallow type. The percentage of infection was also relatively small in skinned roots and in roots with broken tips. In the types of wounds, aside from roots bruised at tip and cross section tip (table 7), the two extremes of relative humidity at 12° C. (93 to 98 and 62 to 65 percent) generally limited infection more during the final storage period than did the adjacent intermediate humidities (84 to 86 and 70 to 76 percent). This

tendency was also present during the initial storage (table 6) whenever infection occurred. The factor limiting infection at relative humidities from 93 to 98 percent was probably suberization and possibly periderm formation during the latter part of the final storage period. At 62 to 65 percent humidity there was a horny layer formed over a cut surface that tended to limit infection unless the roots were subsequently stored at high humidity (above 90 percent). The relative humidities employed during the subsequent storage were 58, 67, 84, and 87 percent.

CONCLUSIONS

The following conclusions may be drawn in regard to the effects of temperature, humidity, types of wounding, suberization, and periderm formation on infection of sweetpotatoes by *Rhizopus*. (1) Since 12° C. is near the lower temperature limit for suberization and periderm formation and since these processes apparently occur only at relative humidities between 90 and 100 percent (10 and 11 days were required for suberization to take place at 12° and a relative humidity of 94 percent and 7 days at 96 percent, and the shortest period at which periderm formation was observed to occur at this temperature was 25 days), infection by *Rhizopus* takes place readily at this temperature. (2) Since suberization and periderm formation take place more rapidly as the temperature is raised above 12° to at least 32°, infection by *Rhizopus* tends to be limited with the rise in temperature within the limits specified. It has been shown by earlier work (*11*) that as the temperature is raised above 33° infection takes place more readily and occurs even in unwounded roots. The factors involved in this increased infection have not been determined. Whether the slowing up of the healing processes at temperatures above 32° is a factor in increased infection is not known. (3) Humidity is more of a limiting factor in infection at 23° than at 28° and 29° because if its effect on suberization and periderm formation. (4) Bruising, because of its effect on suberization and periderm formation, permits of infection fairly readily at most of the temperatures (within the limits of those discussed) and at most humidities except at 28° to 29°, where there was some irregularity of infection at the various relative humidities. (5) Infection is correlated more or less with the extent and degree of wounding. (6) While low humidities at temperatures of 12° and 23° inhibit infection in freshly wounded roots because of their effect on the pathogenes, infection takes place readily when the roots are returned to humidities above 90 percent.

SHRIVELING, SUPERFICIAL GROWTH OF PENICILLIUM, AND INVASION OF WOUNDED AREAS OF SWEETPOTATOES BY CERTAIN FUNGI AS RELATED TO HEALING, TEMPERATURE, AND HUMIDITY

In connection with the *Rhizopus* studies, observations were made on the effect of 10 days' initial storage of wounded sweetpotatoes at different temperatures and relative humidities on shriveling, superficial growth of *Penicillium*, and invasion of wounded areas by certain fungi during 47 days of subsequent storage at 10° C. and 84 percent relative humidity. The observations were made on the following types of wounding: Longitudinal shallow, longitudinal medium, longitudinal half, cross section medium, and cross section half (table 8).

TABLE 8.—*Shriveling, superficial growth of Penicillium, and invasion by certain fungi in wounded sweetpotatoes initially stored for 10 days at different temperatures and relative humidities and subsequently for 47 days at 10° C. and 84 percent relative humidity*

Initial storage		Data on roots wounded as indicated															
		Longitudinal shallow						Longitudinal medium						Longitudinal half			
Temperature (° C.)	Relative humidity	Roots used	Roots showing *Penicillium* on cut surface	Roots showing cut areas sunken	Depth of sunken areas	Roots invaded	Average depth of decay	Roots used	Roots showing *Penicillium* on cut surface	Roots showing cut areas shriveled	Degree of shriveling	Roots invaded	Average depth of decay	Roots used	Roots showing *Penicillium* on cut surface	Roots showing shriveling	Degree of shriveling
	Percent	*Number*	*Number*	*Number*	*Mm*	*Number*	*Mm*	*Number*	*Number*	*Number*		*Number*	*Mm*	*Number*	*Number*	*Number*	
35	91	10	0	0	0.0	0	0.0	10	0	1	Slight	0		20	11	20	Very bad.
32	90	10	0	0	0	0	0	10	0	1	do	0		20	0	4	Slight.
29	95	10	0	0	0	0	0	10	1	2	do	1	4.0	20	9	11	Do.
25	92	10	0	0	0	0	0	10	0	0	None	0		19	2	4	Do.
22	94	10	1	1	.2	1	3.0	9	0	0	do	2	3.5	20	9	5	Slight to marked.
19	93	10	1	6	0.5–1	1	3.0	10	0	2	Very slight	1	2.0	19	17	18	Marked.
15	94	10	3	10	.5–3	9	1.2	10	7	8	Marked	8	4.0	12	12	12	Bad.
12	94	10	8	10	1–3	10	1.3	10	7	10	do	9	4.5	(1)			
29	95	10	0	0		0		10	1	2	Slight	1	4.0	20	9	11	Slight.
29	91	10	0	3	.5	0		10	0	1	do	0		20	11	13	Marked.
29	83	10	3	10	.5–1	0		10	1	1	do	2	1.0	20	16	18	Do.
29	74	10	3	10	.5–1	0		10	5	6	Slight to marked	7	2.6	(1)			
29	66	10	1	10	.5–1.5	0		10	6	8	do	3	7.0	(1)			
12	94	10	8	10	1–3	10	1.3	10	7	10	Marked	9	4.5	(1)			
12	84	10	9	10	2–4	10	2.5	8	8	8	Very marked	8	4.0	(1)			
12	77	9	9	9	2–5	8	2.8	4	4	4	do	4	4.0	(1)			
12	62	10	10	10	2–6	10	3.0	3	3	3	do	3	5.0	(1)			

[1] Where there are no data recorded, the roots not infected by *Rhizopus* were discarded before these data were taken.

TABLE 8.—*Shriveling, superficial growth of Penicillium and invasion by certain fungi in wounded sweetpotatoes initially stored for 10 days at different temperatures and relative humidities and subsequently for 47 days at 10° C. and 84 percent relative humidity*—Continued

Initial storage		Data on roots wounded as indicated											
		Cross section medium						Cross section half					
Temperature (° C.)	Relative humidity	Roots used	Roots showing *Penicillium* on cut surface	Roots showing cut areas shriveled	Degree of shriveling	Roots invaded	Average depth of decay	Roots used	Roots showing *Penicillium* on cut surface	Roots showing shriveling	Degree of shriveling	Roots invaded	Average depth of decay
	Percent	*Number*	*Number*	*Number*		*Number*	*Mm*	*Number*	*Number*	*Number*		*Number*	*Mm*
35	91	10	0	1	Marked	1		10	0	7	Slight to bad	6	7.7
32	90	10	0	0	None	0		10	1	3	do	3	7.7
29	95	10	1	1	Slight	3	1.7	10	0	4	Marked	4	9.5
25	92	10	1	0	None	3	1.0	10	4	3	Marked to very marked	4	13.0
22	94	10	1	2	Slight	2	4.5	10	0	1	Slight	2	1.5
19	93	10	1	0	None	2	2.5	10	5	7	Marked	7	5.7
15	94	9	9	9	Marked	10	6.3	10	9	10	Very marked	10	7.3
12	94	8	8	8	do	8	17.0	9	9	9	do	9	7.4
29	95	10	1	1	Slight to marked	3	1.7	10	0	4	Marked	4	9.5
29	91	10	0	2	do	2	3.0	10	0	5	Slight to marked	4	8.5
29	83	10	2	2	Slight	0		10	5	9	do	7	3.4
29	74	10	5	5	Slight to marked	4	2.3	9	9	9	Marked to very marked	9	6.8
29	66	9	7	6	do	3	10.0	9	9	9	do	9	16.0
12	94	8	8	8	Marked	8	17.0	9	9	9	Very marked	9	7.4
12	84	9	9	9	do	9	12.0	4	4	4	do	4	17.0
12	77	4	4	4	do	4	4.5	2	2	2	do	2	12.0
12	62	4	4	4	Very marked	4	14.0	3	3	3	Bad	3	14.0

Temperature during the initial storage period was a factor affecting shriveling, largely, it is believed, because of its influence on suberization and periderm formation. At temperatures of 22° C. and above (combined with high humidity) there was less shriveling than at 12°, 15°, and sometimes 19° (combined with high humidity), although the saturation deficit was smaller at these temperatures than at the higher temperatures.

Data taken from the experiments under discussion show that suberization and periderm formation take place in a shorter time at temperatures from 22° to 32° than at 19° C. and below. Considerable evaporation of moisture probably took place at the lower temperatures before suberization and periderm formation occurred. There was a tendency for the number of roots showing shriveling to increase with the lowering of the relative humidity at 29°. This increase in shriveling was correlated with the lowering of the relative humidity and the corresponding increase in time required for suberization and periderm formation. Practically all the roots, irrespective of the type of wounding, showed sunken areas or shriveling [10] at all the relative humidities at 12°.

The presence of *Penicillium* and infection were closely associated with conditions of temperature and relative humidity during initial storage favoring shriveling—conditions that either retarded or did not permit of suberization and periderm formation.

Out of 156 isolations made from infected roots, 47.4 percent yielded *Penicillium*, 21.8 percent *Fusarium*, 5.8 percent unidentified fungi, 3.9 percent *Alternaria*, 1.9 percent *Botrytis*, and 19.2 percent were sterile. Temperature and humidity did not seem to influence the occurrence of any of these fungi directly or independently of their effect on healing and shriveling. These fungi, except perhaps *Fusarium*, normally are not active sweetpotato pathogenes but invade wounded tissue left unprotected by lack of suberization and periderm formation.

SURFACE ROT

METHOD OF INFECTION

Infection of sweetpotatoes with surface rot (*Fusarium oxysporum*) takes place chiefly through broken secondary roots, sprouts, and wounds produced by skinning. Wounds deeper than those made by skinning, if not properly healed, usually give rise to other types of decay. Infection through secondary roots generally occurs in freshly dug and improperly cured roots. Infection through sprouts is less frequent and probably occurs only when the sprouts are broken off, injured, or killed in some way.

RELATION OF WOUNDING, TEMPERATURE, AND HUMIDITY TO INFECTION IN CURED AND UNCURED SKINNED SWEETPOTATOES

Four experiments were conducted during 2 seasons (1928–29 and 1929–30) in which sweetpotato roots (Yellow Jersey Improved), skinned in 2 or 3 places (skinned areas about 8 to 10 mm in diameter), were stored at different temperatures and relative humidities for 10 days and subsequently stored at a common temperature and relative humidity for varying periods of time.

The results of all 4 experiments were so similar that the data from only 2 are presented (table 9). In one of these experiments, roots

[10] The degree of shriveling noted was only estimated, except where the depth of the shrinkage is given, and must therefore be regarded as only roughly approximate.

taken directly from the field and skinned without curing were stored at the various initial temperatures and relative humidities; in the other experiment, the roots were cured for 10 days at a temperature of 21° to 24° C. and a relative humidity varying from 52 to 82 percent, and were subsequently stored at a temperature of about 10° to 15° and a relative humidity of about 73 percent for 115 days before skinning and initial storage. The initial and subsequent storage temperatures and relative humidities are given in table 9.

In addition to dealing with the relation of skinning to infection in cured and uncured roots, the results recorded in table 9 contain data on unskinned uncured roots that are discussed later (p. 303).

With the exception of the infection that occurred at a temperature of 25° C. and 91 percent relative humidity, there was no infection in uncured skinned roots initially stored at temperatures from 21° to 36.5° combined with high humidity. The writer has no explanation for the heavy infection at 25°. One infection occurred at this temperature in 1 of the other 3 experiments, but none occurred in the other 2 experiments. There was an increase in the percentage of infection as the temperature was lowered from 21° to 12°.

At a temperature of 28° C. infection occurred at relative humidities of 59, 69, and 78 percent; the lower the humidity the higher was the percentage of infection. No infection occurred at this temperature at relative humidities of 90 and 96. At a temperature of 12° a high degree of infection occurred at all the relative humidities. Although it was lower at relative humidities of 86 and 93 percent than at 65 and 75 percent, this difference involved the absence of infection in only one skinned area at each humidity.

TABLE 9.—*Effect of temperature and humidity during 10 days' storage of skinned and unskinned uncured roots and skinned cured roots on the development of surface rot (Fusarium oxysporum) during 154 days' subsequent storage at 15.5° C. and 87 percent relative humidity in the case of uncured roots and 58 days' subsequent storage at 10° and 84 percent relative humidity in the case of the cured roots* [1]

Uncured roots						Cured roots (skinned)			
Initial storage conditions		Skinned		Unskinned		Initial storage conditions		Skinned areas infected	Depth of decay
Temperature (° C.)	Relative humidity	Roots infected	Skinned areas infected	Roots infected	Average lesions per root	Temperature	Relative humidity		
	Percent	*Percent*	*Percent*	*Percent*	*Number*	*°C.*	*Percent*	*Percent*	*Mm*
36.5	95	0	0	0	0.0	35	91	0	0.0
31	95	0	0	0	0	32	90	0	0
28	96	0	0	0	0	29	95	0	0
25	91	60	50	0	0	25	92	0	0
21	91	0	0	60	5.1	22	94	0	0
19	89	60	50	44	2.0	19	93	7	1.0-2
16	89	80	80	70	14.5	15	94	77	.5-5
12	93	100	90	100	19.1	12	94	90	.5-4
28	96	0	0	0	0	29	95	0	0
28	90	0	0	10	.8	29	91	0	0
28	78	40	40	30	1.7	29	83	10	.5
28	69	80	70	90	6.1	29	74	6	.5
28	59	100	100	50	4.4				
12	93	100	90	100	19.1	12	94	90	.5-4
12	86	100	90	100	17.4	12	84	87	.5-5
12	75	100	100	100	36.6	12	77	83	.5-5
12	65	100	100	100	30.0	12	66	90	.5-4

[1] 10 unskinned and 5 skinned uncured and 10 skinned cured roots were stored at each temperature. The skinned uncured roots were each skinned in 2 places, and the skinned cured roots were each skinned in 3 places. In a few instances roots were removed from the experiments following the initial storage because of decay by *Rhizopus*.

The results obtained from cured and uncured skinned roots (table 9) corresponded closely. The percentage of infection at 29° C. and 74 percent relative humidity was less in cured roots (6 percent) than at 28° and 78 percent relative humidity in uncured roots (40 percent). In the third experiment (not in table 9), involving relative humidities of 63, 71, 82, 90, and 96 percent at 29°, infection occurred only at a relative humidity of 63 percent. It will be seen, therefore, that there is some variation in infection at the various humidities at 28° and 29°. The results, however, are consistent in showing that the lower relative humidities involve a greater hazard for infection.

The results of the four experiments at a temperature of 12° C. show heavy infection, ranging from 83 to 100 percent of the wounded areas at all four humidities. There was no consistent relation of infection to the relative humidity at 12°.

RELATION OF TEMPERATURE AND HUMIDITY TO NORMAL INFECTION IN UNCURED SWEETPOTATOES

One experiment was conducted in which uncured unskinned sweetpotatoes were stored at harvest time at 15 different combinations of temperature and relative humidity (table 9) for 10 days and then stored at a common storage temperature of 15.5° C. and a relative humidity of 87 percent for 154 days. The roots were carefully selected for their freedom from wounds and other defects. Most of the infections occurred through broken secondary rootlets, some through slight abrasions of the skin, and some through sprouts. The results correspond closely with those obtained in roots artificially skinned before being exposed to the initial temperatures and humidities. No infection occurred in roots initially stored at temperatures ranging from 25° to 36.5° C. There was a tendency for the percentage of roots infected, as well as the average number of lesions per root, to increase with the lowering of the temperature (combined with high humidity) from 25° to 12°, although infection was heavier at 21° than at 19°. Again, there was a tendency for the percentage of infection, as well as the average number of lesions per root, to increase with the lowering of the relative humidity at 28°, although there was less infection at 59 than at 69 percent relative humidity. One hundred percent of the roots stored at each of the four relative humidities at 12° became infected. There was little relation between the average number of lesions per root and the relative humidity at which they were initially stored, although the average number of lesions per root was larger at the 2 lower humidities than at the 2 higher.

All the results of surface rot experiments show: (1) That the initial 10 days' storage largely governed infection with surface rot; (2) that the temperatures and relative humidities favorable for suberization and periderm formation limited infection; and (3) that the conditions of temperature and relative humidity that retarded or prevented suberization and periderm formation permitted of various amounts of infection with surface rot, depending upon the inhibition of the rate of wound-cork formation.

FUSARIUM END ROT

The rare infection by *Rhizopus* of roots that had been wounded either by cutting or by breaking off one tip end provided an unusual opportunity to study the effect of temporary storage at various

combinations of temperature and relative humidity followed by subsequent storage at a common temperature and relative humidity on healing and on invasion of sweetpotato tissue by fungi such as forms of *Fusarium*, *Penicillium*, *Alternaria*, etc. Infection by *Rhizopus* also was sufficiently limited as a result of temporary storage of roots bruised at one tip end to permit a study of infection as related to temperature and humidity of the initial storage.

Five experiments dealing with different types of wounding and infection by *Rhizopus* were run during a period of two storage seasons (1928–29 and 1929–30). All three types of wounded roots were employed in each of four of the experiments, and roots bruised at tip and roots cut in cross section at tip were used in the fifth experiment. In one of the experiments uncured roots were used; in the other four, cured roots.

The results of three experiments, all run during different periods in the same season and involving roots from the same crop, are shown in tables 10 and 11.

TABLE 10.—*Effect of temperature and humidity during 10 days' storage on invasion and infection by species of Fusarium, Penicillium, etc., of uncured and cured sweetpotatoes wounded at one tip end of the roots* [1]

Initial storage conditions		Bruised tip			Broken tip and cross section tip		
Temperature (° C.)	Relative humidity	Roots used	Roots infected	Total depth of decay [2]	Roots used	Roots infected	Total depth of decay
	Percent	*Number*	*Percent*	*Mm*	*Number*	*Percent*	*Mm*
UNCURED ROOTS (EXPERIMENT 1)							
36.5	95	9	0	0	13	8	85
31	95	9	0	0	20	5	25
28	96	10	0	0	17	0	0
25	91	10	30	62	20	0	0
21	91	10	10	6	19	5	73
19	89	10	0	0	21	24	20
16	89	8	0	0	18	33	27
12	93	9	0	0	20	50	153
28	96	10	0	0	17	0	0
28	90	7	0	0	20	0	0
28	78	9	0	0	20	0	0
28	69	10	0	0	20	15	49
28	59	10	0	0	19	21	59
12	93	9	0	0	20	50	153
12	86	10	0	0	14	29	35
12	75	10	10	30	20	55	71
12	65	5	0	0	20	70	228
CURED ROOTS (EXPERIMENT 2)							
37	93	4	0	0	8	13	40
31	94	4	0	0	10	10	10
29	96	3	0	0	10	20	37
25	94	5	20	12	10	0	0
22	93				15	13	
21	90	4	0	0	5	0	0
15.5	92	3	0	0	10	60	167
12	93	2	0	0	10	90	36
29	96	3	0	0	10	20	37
29	90	5	0	0	8	13	23
29	82	5	0	0	9	0	0
29	71	5	0	0	10	20	10
29	63	5	0	0	10	40	182
12	93	2	0	0	10	90	36
12	85	4	100	45	10	100	157
12	76	5	80	140	10	100	358
12	63				10	100	447

[1] The uncured roots were harvested Oct. 11, 1929, wounded immediately, and stored at various initial temperatures and relative humidities for 10 days, after which they were stored at a temperature of 15.5° C. and a relative humidity of 87 percent for 154 days. The cured roots were harvested at the same time, cured for 10 days at a temperature of 29° and a relative humidity of 74 percent, exposed, after being wounded, to initial temperatures and humidities for 10 days, and then stored for 142 days at 12.5° and a relative humidity of 58 percent.

[2] Total depth of decay includes decay in all roots.

TABLE 11.—*Effect of temperature and humidity during 10 days' storage on invasion and infection by species of Fusarium, Penicillium, etc., of cured sweetpotatoes wounded at the tip end of the roots* [1]

Initial storage conditions		Infection following indicated type of wounding (experiment 3)											
		Bruised tip						Cross section tip					
Temperature (° C.)	Relative humidity	Roots used	Roots infected		Total penetration of decay [2]	Average penetration	Extremes of penetration	Roots used	Roots infected		Total penetration of decay [2]	Average penetration	Extremes of penetration
	Percent	*Number*	*Number*	*Percent*	*Mm*	*Mm*	*Mm*	*Number*	*Number*	*Percent*	*Mm*	*Mm*	*Mm*
31	94	5	1	20	6	1.2	6	5	0	0	0	0.0	0
29	96	5	0	0	0	0	0	5	1	20	9	1.8	9
25	94	3	2	66	11	3.7	5–6	5	0	0	0	0	0
21	93	2	2	100	28	14.0	10–18	5	0	0	0	0	0
17	89	4	4	100	20	5.0	4–7	5	1	20	6	1.2	6
15.5	95	4	4	100	21	5.3	3–8	4	4	100	15	3.8	2–5
12	94							5	5	100	39	7.8	4–19
29	96	5	0	0	0	0	0	5	1	20	9	1.8	9
28	89	4	1	25	13	3.3	13	4	0	0	0	0	0
28	78	3	1	33	7	2.3	7	5	0	0	0	0	0
28	69	5	0	0	0	0	0	5	1	20	3	.6	3
28	62	5	0	0	0	0	0	5	0	0	0	0	0
12	94							5	5	100	39	7.8	4–19
12	85	4	4	100	41	10.3	2–20	6	6	100	218	36.3	4–81
12	76	4	4	100	99	24.8	4–80	5	5	100	228	45.6	7–97
12	64	4	4	100	27	6.8	3–11	2	2	100	19	9.5	8–11

[1] These roots were harvested Oct. 11, 1929, cured 10 days at a temperature of 29° C. and a relative humidity of 74 percent, and stored at a temperature of 10° to 15° and a relative humidity of 65 to 80 percent until Jan. 23, 1930, the date of the initiation of this experiment. The roots were then wounded and stored for an initial period of 10 days at various temperatures and relative humidities and for a subsequent period of 50 days at 13° and 50 percent relative humidity and of 147 days at 15.5° and 87 percent relative humidity.

[2] Total penetration of decay includes decay in all roots.

The data obtained from the two types of wounding, broken tip and cross section tip, were so similar that the results from experiments 1 and 2 were summarized together in table 10. The results from these two types of wounding were consistent in showing either no infection or comparatively small percentages of infection following exposure to initial temperatures above 19° C. combined with high humidity, the highest individual percentage being 20 at 29° and 96 percent relative humidity (cured roots, table 10). As the initial storage temperature fell below 21° there was in most instances a tendency for the percentage of infection to increase (tables 10 and 11). Infection took place readily in roots subjected to all four humidities at a temperature of 12°. The distribution of infection as related to the various initial relative humidities at 28° and 29° was erratic.

The results obtained when the roots were bruised at one of the root tips are not quite so consistent in their relation to the conditions of initial storage, although, in the main, infection took place more readily in roots subjected to initial storage conditions unfavorable for healing. There is a tendency for bruised tissue to dry out in roots that escape infection by *Rhizopus*. It is possible that the drying out may impede the activities of such fungi as *Fusarium*, *Penicillium*, etc., during the period of subsequent storage long enough to permit the process of healing to go on to completion in many roots if the environmental conditions of subsequent storage are favorable.

Out of 193 isolations made from roots infected through these wounded tip ends, 46 percent yielded forms of *Fusarium*, mostly of the *elegans* group; 27 percent, *Penicillium;* 4 percent, *Alternaria;* 1 percent, unidentified fungi; 0.5 percent, *Rhizopus tritici;* 0.5 percent, *R. nigricans;* 1 percent, *Mucor;* and 21 percent were sterile. Most of the lesions resembled fusarium end rot. It is believed that *Penicillium* invasion was largely secondary because it was never the soft watery type characteristic of the rot normally produced in sweetpotatoes by forms of *Penicillium*. It is believed that the foregoing data throw some light on the occurrence of fusarium end rot in storage. Its occurrence has remained more or less obscure largely because most of the efforts to produce it by artificial inoculation have failed. A partial explanation of this failure is probably that following inoculation the roots were stored under conditions that favor healing. The condition that apparently favors infection is a prolonged inhibition of healing. Associated with an inhibition of healing is an apparent deterioration of host tissue in the wounded areas. It is possible that the deteriorated tissue may assist the forms of *Fusarium* in establishing themselves in the host tissue.

JAVA BLACK ROT

RELATION OF TEMPERATURE TO GROWTH OF DIPLODIA TUBERICOLA

To determine the influence of temperature on the growth of *Diplodia tubericola* an experiment was conducted in which 200-ml Erlenmeyer flasks, each containing 40 ml of carrot agar, were inoculated by placing at the center of the agar a small bit of fungus growth, including pycnidia and spores, from a corn-meal culture of *D. tubericola*. Ten flasks were placed at each of a number of temperatures (table 12), from 2° to 42° C. All measurements of the diameter of

the colonies taken before the growth reached the borders of the agar were taken with calipers that were extended down into the flasks.

No growth occurred in 16 days at temperatures of 40° or 42° C., nor in 35 days at 8°. The maximum amount of growth in 1 day occurred at 31°. Only slightly less growth occurred at 29°. There was a gap of 6° between temperatures 31° and 37°. It is possible that the rate of growth may have been greater at temperatures within this gap had such temperatures been employed. The fact that the amount of growth at 29° was nearly as great as at 31° and that there was a marked drop in the amount of decay as the temperature rose to 37° would seem to indicate that 31° was near the optimum temperature for the growth of *Diplodia tubericola* on culture media. The retardation of growth was pronounced as the temperature rose above 31° or fell below 29°.

TABLE 12.—*Influence of temperature on the growth of Diplodia tubericola on carrot agar*

Temperature (° C.)	Average area of colonies after—			
	1 day	3 days	7 days	11 days
	Mm²	*Mm²*	*Mm²*	*Mm²*
42	0	0	0	[1] 0
40	0	0	0	[1] 0
37	800			
31	2,099			
29	2,004			
25	866			
21.5	547			
19	262			
15	[2]	203		
13	0	[2]	210	
8	0	0	0	[3] 0

[1] There was no growth at 40° and 42° in 16 days.
[2] Growth just started.
[3] There was no growth at 8° or below in 35 days.

RELATION OF WOUNDING TO INFECTION

No experiments were conducted that were especially designed to determine the effect of wounding on infection by *Diplodia tubericola* or to test the capacity of the pathogene to penetrate the unbroken skin. In the experiments discussed below, the uninjured skin of many roots was exposed to inoculum of the pathogene under conditions that readily induce infection in wounded tissue. The results obtained show that normally the fungus does not cause infection through the uninjured skin. *D. tubericola* sometimes invades lesions produced by species of *Rhizopus* and by *Mucor racemosus*, and possibly other lesions.

TECHNIC EMPLOYED IN STUDY OF RELATION OF TEMPERATURE AND HUMIDITY TO INFECTION BY DIPLODIA TUBERICOLA IN WOUNDED SWEETPOTATOES

The technic employed in the infection experiments with *Diplodia tubericola* and *Sclerotium bataticola* was developed as a result of the experience gained in the experiments already discussed.

Unlike *Rhizopus tritici*, *R. nigricans*, and *Fusarium oxysporum*, *Diplodia tubericola* commonly is not found associated with sweetpotatoes grown in the vicinity of Washington, D. C. Only an occasional infection has been observed to occur normally. In order to

insure uniform infection, wounded roots (cross section tip) [11] were dipped in a macerated culture of the fungus grown on corn meal. This method of inoculation insured the presence of the pathogene on the wounded area and at the same time avoided rewounding during the process. Such a precaution was necessary in the wound-healing studies that follow. This method of wounding lent itself admirably to the study of the environmental conditions under which infection occurred. Infection by the species of *Rhizopus* in this type of wounding was rare, and the slowness with which invasion by *Fusarium* occurred enabled such pathogenes as *Diplodia tubericola* and *Sclerotium bataticola*, when present, to establish themselves in such unhealed wounds whenever the temperature and humidity were favorable.

INFECTION IN SWEETPOTATOES INOCULATED WITH DIPLODIA TUBERICOLA WHEN FRESHLY WOUNDED AND IN THOSE INOCULATED AFTER 10 DAYS' STORAGE AT VARIOUS TEMPERATURES AND HUMIDITIES FOLLOWING WOUNDING

Five experiments were conducted which were designed to yield information regarding the following questions: (1) Do sweetpotatoes change during the storage season in their susceptibility to infection by *Diplodia tubericola?* (2) What conditions of temperature and humidity favor infection of freshly wounded roots? (3) What effect does 10 days' storage of wounded roots at various temperatures and relative humidities before inoculation have on infection during subsequent storage following inoculation?

The Yellow Jersey variety was used in all five experiments. The treatment the roots received before and during the experiments was, briefly, as follows:

In experiment 1 the roots were harvested October 6, 1928, cured for 10 days at a temperature of 22° to 26° C. and a relative humidity of 50 to 82 percent, and then stored at a temperature of 10° to 15° and a relative humidity of 70 to 85 percent until February 13, 1929, when the experiment was started. The roots were inoculated when freshly wounded (cross section tip) and 10 roots each were stored at 15 combinations of temperature and relative humidity for 46 days.

Experiment 2 was started February 25, 1929, with roots from the stock used in experiment 1. (1) A quantity of roots were inoculated when freshly wounded (cross section tip) and stored 10 each at the 15 combinations of temperature and relative humidity given in table 13 and in addition at a temperature of 15.5° C. and a relative humidity of 87 percent and at 21° and 89 percent relative humidity for 45 days. (2) A quantity of roots were wounded, then stored 10 each at 15 combinations of temperature and relative humidity for 10 days, and then inoculated; after inoculation these roots were further treated as follows: (*a*) Roots from each initial temperature and relative humidity were returned to the conditions of temperature and relative humidity from which they came for 35 days' further storage, and (*b*) roots from each initial storage condition were stored at 15.5° and 87 percent relative humidity and at 21° and 89 percent relative humidity for 35 days.

In experiment 3 roots from the stock used in experiments 1 and 2 were wounded March 5, 1929, by cutting a thin slice (longitudinal section shallow p. 295) from the side of each root. After wounding, the procedure was the same as in experiment 2, except that the subse-

[11] The roots were wounded by cutting off one tip end of each root at a point where the root was about 1 cm in diameter.

quent storage at 15.5° C. and 87 percent relative humidity was omitted and the storage at the other conditions lasted only 26 days.

In experiment 4 roots harvested October 11, 1929, were wounded and inoculated immediately without curing, and stored 10 each at the various temperatures and relative humidities shown in table 13, for 61 days.

In experiment 5 uncured roots from the stock used in experiment 4 were subjected to the same treatment and procedure, except the duration of subsequent storage (table 13), as those in experiment 2, five roots being used at each storage condition.

TABLE 13.—*Infection of sweetpotatoes by Diplodia tubericola during various periods of storage following different treatments* [1]

Initial storage conditions		Infection in roots inoculated—							
		When freshly wounded (after inoculation roots stored for 21 days at initial storage conditions only)		After 10 days at initial storage conditions following wounding					
				Subsequent storage for 51 days at conditions same as initial		Subsequent storage for 51 days at 15.5° C. and 87 percent relative humidity		Subsequent storage for 51 days at 21° C. and 89 percent relative humidity	
Temperature (° C.)	Relative humidity	Roots infected	Total depth of decay	Roots infected	Total depth of decay	Roots infected	Total depth of decay	Roots infected	Total depth of decay
	Percent	*Percent*	*Mm*	*Percent*	*Mm*	*Percent*	*Mm*	*Percent*	*Mm*
36.5	95					0	0	0	0
31	95	80	218	0	0	0	0	0	0
28	96	80	87	0	0	0	0	0	0
25	91	80	25	0	0	20	2	0	0
21	91	80	17	0	0	20	40	0	0
19	89	100	25	20	8	40	16	0	0
16	89	60	14	60	13	60	17	40	115
12	93	40	6	0	0	60	12	40	170
28	96	80	87	0	0	0	0	0	0
28	90	100	50	0	0	0	0	20	3
28	78	60	13	0	0	20	17	20	15
28	69	60	105	0	0	0	0	40	10
28	59	40	127	20	25	[2] 50	33	40	42
12	93	40	6	0	0	60	12	40	170
12	86	60	8	40	9	80	18	20	45
12	75	100	23	80	40	100	54	100	63
12	65	60	61	100	74	[3] 66	46	100	91
Check (roots inoculated when freshly wounded)						100	103	80	114

[1] 5 roots were used for each condition of storage except in the cases noted. All roots were wounded by cutting off the tip end.
[2] 6 roots were used instead of 5.
[3] 3 roots were used instead of 5.

Whenever the treatment and storage conditions were comparable the results of all five experiments were so similar that only those of experiment 5 are presented in table 13.

The results of the five experiments, together with some [12] discussed later (p. 311), show that if cured and uncured roots are wounded in the same manner and the subsequent treatment as to inoculation and storage is the same there is no apparent difference in their susceptibility to infection by *Diplodia tubericola*. Infection occurred under

[12] The results from these later experiments were obtained from cured roots from the same stock as the uncured roots in experiment 5. One of these experiments was started Nov. 26, 1929, following curing, and one Jan. 13, 1930.

the same conditions and in similar proportions in cured and uncured roots.

A certain percentage of infection by *Diplodia tubericola* occurred at all conditions of temperature and humidity in roots inoculated when freshly wounded (table 13). The tendency for percentage of infection to drop at temperatures below 19° C. was also evident in the other experiments (not shown in table 13). There was a tendency for the total depth of decay to increase with the rise in temperature from 12° to 31°.

There seems to be little relation of humidity to the percentage of infection at 28° C. in roots inoculated when freshly wounded, although the results in table 13 suggest a possible relation. In 2 of the 5 experiments there was no infection at a relative humidity of 96 percent, and about an equal percentage of infection occurred at the other humidities. At a temperature of 12° there was a tendency for the percentage of infection as well as the total depth of decay to increase with the lowering of the relative humidity, particularly in the early stages of infection. In this experiment and in one other (table 13) the percentage of infection increased with the lowering of the relative humidity from 93 to 73 percent, then dropped off as the humidity fell to 65 percent. A temperature of 12° has not been found very favorable to the continuation of decay by *Diplodia tubericola*. In a large percentage of the cases where infection began with this pathogene, the organism was displaced by species of *Fusarium* and *Penicillium*.

In roots wounded and stored for 10 days at the various temperatures and humidities, then inoculated and returned to the temperatures and humidities from which they came for 51 days' further storage, infection had a very definite relation to the conditions of initial storage that are either unfavorable to suberization and periderm formation or retard their formation. No infection occurred at temperatures of 21°, 25°, 28°, and 31° C. combined with high humidity. Passing from a temperature of 21° to 19° and 16°, the percentage of infection as well as the total penetration increased. At a temperature of 12° there was no infection at a relative humidity of 93 percent. In 3 out of the 4 other experiments (data not included in table 13) there was infection at 12° (combined with the highest humidity employed); in 2 of these 3 there was no infection at temperatures above 12°. Infection took place readily at the three lower humidities at 12°. At 28° only relative humidities of 59 percent in this experiment (table 13) and 63 percent in another experiment, of the 5 employed, were sufficiently unfavorable to healing to permit of infection. Infection was obtained at 59 percent relative humidity in two other experiments.

If subsequent storage, following 10 days' initial storage at various temperatures and humidities, influences infection and decay, it does so in the case of roots stored at initial combinations of temperature and relative humidity that either prevented or retarded suberization or periderm formation. Subsequent storage conditions favorable to cork formation usually resulted in less infection than conditions unfavorable to cork formation in roots initially stored under conditions of temperature and humidity unfavorable to healing. The results have

been consistent in showing that whenever the conditions of temperature, humidity, and time were such as to permit cork formation to go on to completion before the roots were inoculated infection did not result under the conditions of subsequent storage employed in these experiments. Storage for 10 days at temperatures of 28°, 31°, and 36.5° C. combined with high humidity at all the conditions of subsequent storage prevented infection. There was one infection at 25°, but decay made scarcely any progress. A subsequent storage at 15.5° and 87 percent relative humidity following initial storage at temperatures of 19°, 16°, and 12° (combined with high humidity) showed a higher percentage of infection, with one exception (table 13), than did roots that were returned to these initial temperatures or were subjected to subsequent storage at 21° and 89 percent relative humidity. In experiment 2 (not shown in table 13) a higher percentage of infection resulted during subsequent storage at 21° and 89 percent relative humidity. There was less infection (table 13) in roots initially stored at the various humidities at a temperature of 28° followed by subsequent storage under these same conditions than in roots with the same initial storage followed by subsequent storage at 15.5° and 87 percent relative humidity and at 21° and 89 percent relative humidity. In experiment 2 (not shown in table 13) there was no infection during subsequent storage at various relative humidities at 28° following an initial storage under the same conditions, except at 63 percent relative humidity, the lowest employed. With certain exceptions, there was a tendency for infection and decay to increase with the lowering of the relative humidities both at 28° and 12° in roots subsequently stored at 15.5° and 21°. In roots from the various initial temperatures and humidities the total decay was greater in the main in those subsequently stored at 21° than at 15.5°.

RELATION OF EXTREMES OF TEMPERATURE AND HUMIDITY DURING INITIAL STORAGE OF WOUNDED CURED SWEETPOTATOES TO INFECTION BY DIPLODIA TUBERICOLA DURING SUBSEQUENT STORAGE

Two experiments were conducted to compare the effects of 10 days' initial storage of wounded sweetpotatoes at high temperatures and relative humidities with those at low temperatures and humidities, during a subsequent storage at various temperatures and humidities. In experiment 1 Yellow Jersey roots were wounded (cross section tip) and stored for 10 days at 2 combinations of temperature and relative humidity (some at 29° C. and 97 percent and some at 13° and 69 percent), then inoculated with *Diplodia tubericola* and stored at 15 combinations of temperature and relative humidity (table 14), together with some roots inoculated when freshly wounded. Experiment 2 included the Puerto Rico variety in addition to Yellow Jersey. The initial storage temperatures and relative humidities were 26° and 92 percent and 13° and 59 percent in the case of Yellow Jersey, and 29° and 97 percent and 13° and 59 percent in the case of Puerto Rico. The subsequent storage temperature of 37° was omitted in experiment 2. Aside from the exceptions mentioned the two experiments were similar. The average results obtained from the Yellow Jersey variety in the two experiments are presented in table 14.

TABLE 14.—*Relation of temperature and humidity to infection and decay of sweetpotatoes (Yellow Jersey) by Diplodia tubericola during 10 to 12 days' storage following different treatments* [1]

Final storage conditions		Roots inoculated when freshly wounded		Roots wounded and initially stored for 10 days at—			
				13° C. and relative humidities of 59 and 69 percent and then inoculated		26° and 29° C. and relative humidities of 92 and 97 percent and then inoculated	
Temperature (° C.)	Relative humidity	Roots infected	Average depth of decay	Roots infected	Average depth of decay	Roots infected	Average depth of decay
	Percent	*Percent*	*Mm*	*Percent*	*Mm*	*Percent*	*Mm*
37	93	20	0.6	20	0.6	0	0
30-31	94	90	17.0	90	25.0	0	0
29	97	100	26.0	70	29.0	0	0
25	92	80	15.0	90	23.0	0	0
22	91-95	60	18.0	60	5.0	0	0
18-19	88-94	30	.9	60	2.0	0	0
15.5	91-92	0	0	40	4.0	0	0
12	93-94	0	0	10	.3	0	0
29	97	100	26.0	70	29.0	0	0
29	89-90	90	41.0	80	32.0	10	9
29	80	100	38.0	100	27.0	0	0
29	69-72	80	17.0	80	20.0	10	8
29	60-63	50	24.0	60	24.0	0	0
12	93-94	0	0	10	.3	0	0
12	85	0	0	0	0	0	0
12	76-78	0	0	0	0	0	0
12	63-64	0	0	0	0	0	0

[1] The results recorded in this table except at 37° C. are the averages of two experiments. Five roots were used for each treatment and condition of temperature and relative humidity in each experiment. Results were taken after 10 and 12 days of final storage, respectively.

The initial storage of wounded roots for 10 days at high temperatures and high relative humidities (26° and 29° C. and 92 and 97 percent relative humidity, respectively) before inoculation excluded infection except at two conditions of subsequent storage (29° and a relative humidity of 89 to 90 percent, and 29° and a relative humidity of 69 to 72 percent) and here only 1 root out of 10 was involved in each instance (table 14). In roots initially stored at a low temperature and low humidity (13° and 59 and 69 percent) infection occurred at all subsequent storage conditions of temperature and relative humidity, except the three lowest humidities at 12°, within 10 to 12 days. In roots inoculated when freshly wounded infection occurred at temperatures of 18° and above, but not at 12° and 15.5°, in 10 to 12 days' subsequent storage. From the occurrence of infection and the amount of decay, it will be seen that there is no significant difference in susceptibility between roots inoculated when freshly wounded and roots inoculated after 10 days' storage at low temperature and relative humidity following wounding.

Results similar to the foregoing were obtained with roots of the Puerto Rico variety, wounded and initially stored for 10 days, in one instance at a temperature of 29° C. and relative humidity of 97 percent and in another at 13° and 59 percent relative humidity, followed by inoculation with *Diplodia tubericola* and 12 days' subsequent storage at 14 combinations of temperature and relative humidity.

RELATION OF TEMPERATURE TO RATE OF DECAY

The results obtained during 10 to 12 days' storage at various temperatures following inoculation from (1) roots inoculated when freshly wounded and (2) roots inoculated after 10 days' storage at a

temperature of 13° C. and relative humidities of 59 and 69 percent illustrate the effect of temperature on rate of decay (table 14 and fig. 1). There is not much difference in the rate of decay in roots with the two types of treatment, although the rate is as great or greater at all temperatures at which infection occurred, except at 22°, in roots subjected to 10 days' initial storage at 13° and 59 and 69 percent relative humidity (fig. 1, *B*) than in roots inoculated when freshly wounded (fig. 1, *A*). There was some variation in individual experiments in the amount of decay that took place at a given temperature, but the average of the two experiments following a given type of treatment produces fairly uniform curves. If averages of the two types of treatment are taken, a still smoother curve is obtained (fig. 1, *C*). The greatest amount of decay occurred at 29° in the experiments reported in table 14, but in another experiment it occurred at 31° (table 13). As the temperature was raised above or lowered below 29° the rate of decay rapidly declined.

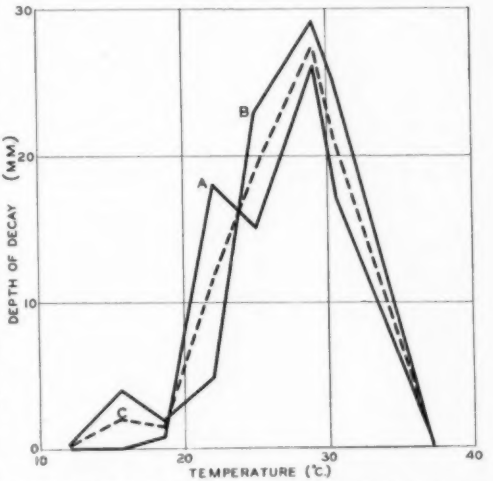

FIGURE 1.—Depth of decay in sweetpotatoes caused by *Diplodia tubericola* at various temperatures: *A*, Roots inoculated when freshly wounded; *B*, wounded roots stored for 10 days at 13° C. and at 59 and 69 percent relative humidity before inoculation; *C*, average of *A* and *B*.

The data in table 15, obtained from one of the experiments discussed in connection with table 14, illustrate roughly the rate at which infection and decay by *Diplodia tubericola* occur at temperatures of 12°, 15.5°, and 17.7° C. following preliminary treatment and inoculation, as indicated. It will be noted that decay takes place very slowly, the average penetration being slight even after 44 days' storage at these temperatures. The slowness in the advance of decay is due in part, it is believed, to the retarded activity of the pathogene at these temperatures. *Diplodia tubericola* is often displaced by other organisms, especially *Fusarium*, at these temperatures, particularly at 12°. The high average depth of decay (13 mm) after 44 days' storage at 12° and 63 percent relative humidity in roots inoculated after wounding and 10 days' storage at 13° and 59 percent relative humidity was due to deep decay (55 mm) in one root rather than to a consistent advance in all the roots. Incidentally it will be noted that the roots with an initial storage temperature of 26° and a relative humidity of 92 percent showed scarcely any infection after 44 days' storage at the temperatures 12°, 15.5°, and 17.7° and the various relative humidities at 12°, or after 16 days' further storage at 21° and 89 percent relative humidity. Nor did these roots show signs of infection by other organisms.

TABLE 15.—*Relation of time, temperature, and humidity to infection and decay of sweetpotatoes (Yellow Jersey) during storage following different treatments*

Storage [1] conditions subsequent to inoculation		Effect of indicated storage period on infection and decay in roots inoculated—																	
		Immediately following wounding								Following wounding and 10 days' storage at 13° and 59 percent relative humidity								Following wounding and 10 days' storage at 26° and 92 percent relative humidity [2]			
		12 days		20 days		44 days		60 days		12 days		20 days		44 days		60 days		44 days		60 days	
Temperature (° C.)	Relative humidity	Infection	Average depth of decay	Infection	Average depth of decay	Infection	Average depth of decay	Infection	Average depth of decay	Infection	Average depth of decay	Infection	Average depth of decay	Infection	Average depth of decay	Infection	Average depth of decay	Infection	Average depth of decay	Infection	Average depth of decay
	Percent	*Percent*	*Mm*	*Percent*	*Mm*	*Percent*	*Mm*	*Percent*	*Mm*	*Percent*	*Mm*	*Percent*	*Mm*	*Percent*	*Mm*	*Percent*	*Mm*	*Percent*	*Mm*	*Percent*	*Mm*
17.7	88	40	1	80	2.4	80	2.2	100	3	40	1	80	2.6	100	3	100	3	0	0.0	0	0
15.5	92	0	0	20	.6	60	1.6	100	2.3	40	2	80	3.4	100	3	100	3	0	0	0	0
12.0	93	0	0	0	0	20	.8	100	3	0	0	25	.8	75	3.5	100	5	0	0	0	0
12.0	85	0	0	0	0	20	.4	100	3	0	0	40	4.6	75	3	100	5	0	0	0	0
12.0	76	0	0	0	0	100	3.0	100	3	0	0	0	0	80	5	100	17	0	0	0	0
12.0	63	0	0	0	0	100	3.0	100	4	0	0	20	.2	100	.13	100	13	20	.6	20	1

[1] During the interval between 44 and 60 days, all the roots were stored at 21° C. and 89 percent relative humidity.
[2] There was no infection after storage periods of 12 or 20 days.

TEMPERATURE LIMITS OF INFECTION

No experiments designed to determine the exact temperature limits of infection for *Diplodia tubericola* were conducted.

The slowness of infection and decay at 12° C., and the fact that *Diplodia tubericola* is largely displaced by other organisms and that there was no growth on carrot agar at 8° after 35 days' storage (table 12) indicate that 12° is near the lower temperature limit of infection and decay for this fungus.

The slowness in rate of decay by this fungus at 37° C. and the fact that there was no growth of the pathogene in 16 days at 40°(table 12) show that 37° is near the maximum temperature for infection and decay.

EFFECT OF DURATION OF INITIAL STORAGE OF WOUNDED SWEETPOTATOES ON INFECTION BY DIPLODIA TUBERICOLA DURING SUBSEQUENT STORAGE

An experiment was conducted in which roots of the Yellow Jersey and Nancy Hall varieties were wounded as in the preceding cases, stored at a temperature of 25.5° C. and a relative humidity of 94 percent for various periods, then inoculated with *Diplodia tubericola* and returned to the same temperature and relative humidity for 13 and 22 days' further storage. The initial storage periods for the Yellow Jersey were 2, 4, 6, 8, and 10 days, respectively, and for Nancy Hall 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 days, respectively. In order to use the same inoculum for all the roots, those for the 10-day initial storage period were wounded first, those for the 9-day period next, etc., each lot being wounded and stored on successive days in the order of time assigned to the initial storage. At the end of the various initial storage periods all the roots, including the freshly wounded ones, were inoculated at the same time with the same inoculum.

No infection occurred in either Yellow Jersey or Nancy Hall roots subjected to an initial storage of more than 4 days (table 16).

TABLE 16.—*Effect of duration of initial storage of wounded sweetpotatoes followed by inoculation with Diplodia tubericola on infection and decay during 2 periods of subsequent storage* [1]

Duration of initial storage (days)	Infection and decay in the Nancy Hall variety after storage periods indicated								Infection and decay in Yellow Jersey variety after storage period indicated			
	Initial, 29° C. and 96-percent relative humidity; subsequent, 28° and 89 percent relative humidity				Initial and subsequent, 25.5° C. and 94 percent relative humidity				Initial and subsequent, 25.5° C. and 94 percent relative humidity			
	13 days		28 days		13 days		22 days		13 days		22 days	
	Roots infected	Depth of decay	Roots infected	Depth of decay	Roots infected	Depth of decay	Roots infected	Depth of decay	Roots infected	Depth of decay	Roots infected	Depth of decay
	Percent	*Mm*	*Percent*	*Mm*	*Percent*	*Mm*	*Percent*	*Mm*	*Percent*	*Mm*	*Percent*	*Mm*
0	100	153	100	[2] 149	100	55	100	58	80	15	100	17
1	80	29	100	33	80	14	100	24				
2	0	0	20	2	40	5	60	13	20	4	60	7
3	0	0	0	0	20	2	60	10				
4 [3]	0	0	0	0	0	0	0	0	0	0	20	2

[1] 5 roots were used for each time interval.

[2] The apparent decrease in depth of decay in 28 days as compared to 13 days is due to error of measurement. The measurements taken after 13 days were from the outside of the root and those taken after 28 days were from the interior after the roots had been halved. However, there was no advance of decay after 13 days.

[3] There was no infection after initial storage periods of 5 days or more.

In another experiment, roots of the Nancy Hall variety were stored for periods of 1 to 10 days at a temperature of 29° C. and a relative humidity of 96 percent and then inoculated and stored at 28° and 89 percent relative humidity for 13 and 28 days (table 16). The wounding and inoculation were made in the same manner as in the preceding experiment. No infection occurred in roots that had had an initial storage period longer than 2 days.

CHARCOAL ROT

RELATION OF TEMPERATURE TO GROWTH OF SCLEROTIUM BATATICOLA ON CARROT AGAR

In order to determine the temperature limits and optimum temperature for growth of *Sclerotium bataticola*, as well as the rate of growth at various temperatures, ten 200-ml Erlenmeyer flasks, each containing 40 ml of carrot agar, were inoculated with *S. bataticola* and stored at each of 11 temperatures (table 17). The maximum amount of growth in 1 day occurred at a temperature of 31° C. As the temperature rose above 31° or fell below it the rate of growth rapidly declined. *S. bataticola* showed slight growth in 3 out of 10 flasks at 42° in 16 days and slight growth in 2 out of 10 flasks at 8° in 35 days in contrast to an absence of growth by *Diplodia tubericola* at these temperatures in the same periods.

TABLE 17.—*Influence of temperature on the growth of Sclerotium bataticola on carrot agar*

Temperature (° C.)	Average area of colonies after—				Temperature (° C.)	Average area of colonies after—			
	1 day	3 days	7 days	11 days		1 day	3 days	7 days	11 days
	Mm²	*Mm²*	*Mm²*	*Mm²*		*Mm²*	*Mm²*	*Mm²*	*Mm²*
42.0	0	0	0	[1] 0	21.5	53			
40.0	0	0	0	[1] 0	19.0	[2]	1,005		
37.0	511				15.0	0	[2]	3,341	
31.0	2,100				13.0	0	0	210	
29.0	580				8.0	0	0	0	[3] 0
25.0	207								

[1] Growth had just started in 3 out of 10 flasks at 42° C. and in 1 out of 10 flasks at 40° C. in 16 days.
[2] Growth just started.
[3] There was slight growth in 2 flasks out of 10 at 8° C. in 35 days.

RELATION OF WOUNDING TO INFECTION

Sclerotium bataticola seems quite dependent upon wounds for infection. It sometimes invades lesions of mottled necrosis, *Rhizopus* soft rot, lesions produced by *Mucor racemosus*, and possibly other lesions. Injury by heat, such as that produced in the field when the roots lie in the sun, seems to open the way for infection in fields heavily infested with *S. bataticola*. However, mechanical wounding remains the major preliminary condition essential to infection. If the unbroken skin is exposed to inoculum of *S. bataticola*, infection rarely if ever results. This is shown by the infection experiments discussed below, conducted under the same conditions and involving the same technic as those carried on in connection with *Diplodia tubericola*.

INFECTION BY SCLEROTIUM BATATICOLA IN SWEETPOTATOES INOCULATED WHEN FRESHLY WOUNDED AND IN THOSE INOCULATED AFTER 10 DAYS' STORAGE AT VARIOUS TEMPERATURES AND HUMIDITIES FOLLOWING WOUNDING

The materials (except the pathogene) and the technic employed in these experiments (five in number) were the same as in the experiments with *Diplodia tubericola*. The experiments were run concomitantly and under the same conditions of temperature and relative humidity.

The results of the five experiments were so similar whenever the conditions of treatment and storage were parallel that the results of only one are presented (table 18). In this experiment uncured roots obtained directly from the field at harvest time (Oct. 10, 1929) were used. One lot was held for 10 days at 15.5° C. and 87 percent relative humidity (noncuring condition), then wounded and inoculated with *Sclerotium bataticola*. Five of these roots (roots inoculated when freshly wounded) were then stored 21 days at each of the 15 combinations of initial temperature and relative humidity given in table 18. Another lot of roots were wounded immediately after harvesting, and part of them were stored for 10 days at each of the 15 initial temperatures and relative humidities given in table 18. At the end of this 10 days' storage all the roots were inoculated. Five of these roots from each initial storage condition were stored as follows: (1) For 21 days at each of the initial storage conditions; (2) for 51 days at 15.5° and 87 percent relative humidity; and (3) for 51 days at 21° and 89 percent relative humidity. Roots inoculated when freshly wounded were also stored under the two last-mentioned conditions of storage as checks. The same inoculum was used throughout, and all the roots were inoculated at one time.

TABLE 18.—*Infection of sweetpotatoes by Sclerotium bataticola during various periods of storage following different treatments* [1]

Initial storage conditions		Infection in roots inoculated—							
		When freshly wounded (after inoculation roots stored for 21 days at initial storage conditions)		After 10 days' storage at initial temperatures and relative humidities following wounding					
				Subsequent storage (for 51 days) at conditions same as initial		Subsequent storage (for 51 days) at 15.5° C. and 87 percent relative humidity		Subsequent storage (for 51 days) at 21° C. and 89 percent relative humidity	
Temperature (° C.)	Relative humidity	Roots infected	Total depth of decay	Roots infected	Total depth of decay	Roots infected	Total depth of decay	Roots infected	Total depth of decay
	Percent	*Percent*	*Mm*	*Percent*	*Mm*	*Percent*	*Mm*	*Percent*	*Mm*
36.5	95	----	----	----	0	----	0	20	150
31.0	95	80	307	20	100	0	0	0	0
28.0	96	60	337	0	0	0	0	0	0
25.0	91	80	202	0	0	0	0	0	0
21.0	91	100	262	0	0	20	15	0	0
19.0	89	100	32	0	0	20	8	0	0
16.0	89	60	10	0	0	60	26	80	131
12.0	93	40	7	0	0	100	25	100	310
28.0	96	60	337	0	0	0	0	0	0
28.0	90	80	134	0	0	0	0	0	0
28.0	78	100	346	0	0	40	25	40	48
28.0	69	100	195	40	117	20	3	20	110
28.0	59	80	248	20	115	20	2	80	16
12.0	93	40	7	0	0	100	25	100	310
12.0	86	40	10	20	10	100	46	100	132
12.0	75	0	0	100	43	80	42	100	281
12.0	65	20	3	100	62	80	27	80	138
Check (roots inoculated when freshly wounded)						100	46	100	390

[1] 5 roots were used for each condition of storage except in the case of roots inoculated when freshly wounded and stored at 15.5° C. and 87 percent relative humidity and at 21° C. and 89 percent relative humidity, in which cases 7 and 10 roots, respectively, were used. All roots were wounded by cutting off 1 tip end of each.

In roots inoculated when freshly wounded, infection by *Sclerotium bataticola* took place readily at temperatures from 19° to 31° C., and at all relative humidities at 28° (table 18). At temperatures below 19° (see also table 19) infection was more uncertain and at 12° *Sclerotium* was generally displaced by other organisms, especially species of *Fusarium*. There seems to be little relation between the percentage of infection and relative humidity at either 28° (see also table 19)[13] or 12°.

An initial 10 days' storage of wounded roots at various temperatures and relative humidities altered materially the infection with *Sclerotium bataticola* that occurred when they were inoculated and stored at the same temperatures and relative humidities as the roots inoculated when freshly wounded. With the exception of 1 root at 31° C., no infection occurred in roots stored for 51 days at any of the temperatures combined with high humidity. There was no infection at temperatures of 12° and 16° combined with high humidity in the experiment reported in table 18, but there was in 2 other experiments at 16°, and in 1 of the 2 at 12° (not included in table 18). The handicaps that these conditions impose upon healing and the activities of the pathogene were such that a struggle for supremacy resulted. Some infection occurred at the 2 lowest relative humidities (59 and 69 percent) at 28° and at the 3 lowest humidities (65, 75, and 86 percent) at 12°. These results show that healing is not always sufficiently complete by the end of 10 days' storage at the lower humidities at 12° and 28° to preclude infection.

Judged by the infection with *Sclerotium bataticola* that occurred (table 18) during subsequent storage at 15.5° C. and 87 percent relative humidity and at 21° and 89 percent relative humidity, healing was not always complete by the end of 10 days' initial storage at temperatures of 12° to 21° (combined with high humidity), at the three lowest humidities at 28°, or at any of the humidities at 12°.[14] The rate of decay was consistently higher at a subsequent temperature of 21° than at 15.5°. Infection occurred following subsequent storage at 15.5° after initial temperatures of 12° to 21°, whereas infection occurred following subsequent storage at 21° after initial temperatures of 12° to 16°. These results (at 15.5° and 21°) were almost reversed in another experiment, in which, however, the subsequent relative humidity at 15.5° was 95 percent instead of 87 percent. In wounded roots inoculated with *Sclerotium bataticola* after a day's initial storage at temperatures from 12° to 21°, infection during subsequent storage would seem to depend upon (1) whether wound healing is complete, (2) the stage of healing, and (3) the temperatures and relative humidity of subsequent storage. If wound healing had reached an advanced stage, it would seem possible that it might become complete before infection could occur, especially if the conditions of subsequent storage were favorable to healing; if unfavorable, infection might take place.

[13] The results in table 18 indicate a possible relation between the percentage of infection and relative humidity at 28° C., but when all the results dealing with infection in freshly wounded roots are considered there seems to be no relation.

[14] The one infection in roots with an initial storage at 36.5° C. and a subsequent storage at 21° may easily have been accidental. However, it may have been associated with the same factor that makes sweetpotatoes more susceptible to decay by *Rhizopus tritici* (*11*) at temperatures above 33°.

RELATION OF EXTREMES OF TEMPERATURE AND HUMIDITY DURING INITIAL STORAGE OF WOUNDED CURED SWEETPOTATOES TO INFECTION BY SCLEROTIUM BATATICOLA DURING SUBSEQUENT STORAGE

The two initial storage temperatures and relative humidities in the two experiments reported in table 19 were selected to compare the effects of storing wounded roots at conditions very favorable to healing with the effects of storing at conditions that prevent healing.

TABLE 19.—*Influence of temperature and humidity on infection by Sclerotium bataticola in cured sweetpotatoes inoculated when freshly wounded and in sweetpotatoes wounded and stored for 10 days at each of two combinations of temperature and humidity and then inoculated* [1]

Final storage conditions		Roots inoculated when freshly wounded		Roots wounded and initially stored for 10 days at—			
				13° C. and relative humidities of 59 and 69 percent and then inoculated		26° and 29° C. and relative humidities of 92 and 97 percent and then inoculated	
Temperature (° C)	Relative humidity	Roots infected	Average depth of decay	Roots infected	Average depth of decay	Roots infected	Average depth of decay
	Percent	*Percent*	*Mm*	*Percent*	*Mm*	*Percent*	*Mm*
37	93	80	11	80	13	0	0
30–31	94	90	77	100	50	0	0
29	97	100	87	100	40	20	9
25	92	100	54	90	21	0	0
22	91–95	88	22	100	28	0	0
18–19	88–94	40	9	50	3	0	0
15.5	91–92	30	1	60	6	0	0
12	93–94	0	0	0	0	0	0
29	97	100	87	100	40	20	9
29	89–90	90	64	80	24	10	9
29	80	90	77	90	57	0	0
29	69–72	100	55	90	16	10	7
29	60–63	100	61	70	19	0	0
12	93–94	0	0	0	0	0	0
12	85	0	0	0	0	0	0
12	76–78	0	0	0	0	0	0
12	63–64	0	0	0	0	0	0

[1] The results recorded are the averages of 2 experiments; 5 roots were used for each treatment and combination of temperature and relative humidity. The length of the final storage period in the experiments was 10 and 12 days, respectively.

The material used, aside from the pathogene, the technic employed in setting up the two experiments, and the initial and final storage conditions were the same as those employed in the same type of experiments in connection with Java black rot. The results reported are the average results of the two experiments.

Infection took place under the same conditions of temperature and relative humidity in final storage (table 19) both with roots inoculated when freshly wounded and with wounded roots inoculated after 10 days' storage at a temperature of 13° C. and relative humidities of 59 and 69 percent. The temperature of the storage following inoculation was the controlling factor governing the percentage of infection and the amount of decay. No infection occurred at any of the relative humidities at 12°, whereas at 29° it occurred at all the relative humidities.

Notwithstanding the heavy inoculation following 10 days' initial storage at temperatures of 26° and 29° and at relative humidities of 92 and 97 percent, very little or no infection occurred at any of the

subsequent storage conditions, indicating fairly complete protection due to formation of wound cork at the favorable temperature and relative humidity of the storage chamber.

RELATION OF TEMPERATURE TO RATE OF DECAY

The effect of temperature on the rate of decay is shown by the average depth of decay obtained in cured sweetpotatoes inoculated with *Sclerotium bataticola* when freshly wounded and from roots initially stored for 10 days at a temperature of 13° C. and relative humidities of 59 and 69 percent before inoculation (table 19). In roots inoculated when freshly wounded, the maximum amount of decay during 10 to 12 days' storage at various temperatures occurred at 29° (table 19 and fig. 2, *A*). At temperatures above or below 29°, the rate of decay declined rapidly. A similar curve was obtained in connection with roots subjected to an initial storage at 13° and 59 and 69 percent relative humidity before inoculation and a subsequent storage at various temperatures, except that the maximum amount of decay occurred at 30° to 31° instead of at 29° and that the amount of decay was less at most of the temperatures (table 19 and fig. 2, *B*). Curve *C* in figure 2 was plotted from the average depth of decay at a given temperature in the roots subjected to the two types of treatment. The curve is slightly smoother and the optimum lies between 29° and 30° to 31°. It will be remembered that the optimum temperature for growth of *S. bataticola* is 31°. Unfortunately, a temperature between 31° and 37° was not employed in either the infection or growth experiments; had one been used it is possible that the optimum temperature for growth might have been shifted to a slightly higher temperature. The fact that the rate of decay in some instances has been greater at 29°, and even at 28° (table 18) in one experiment, than

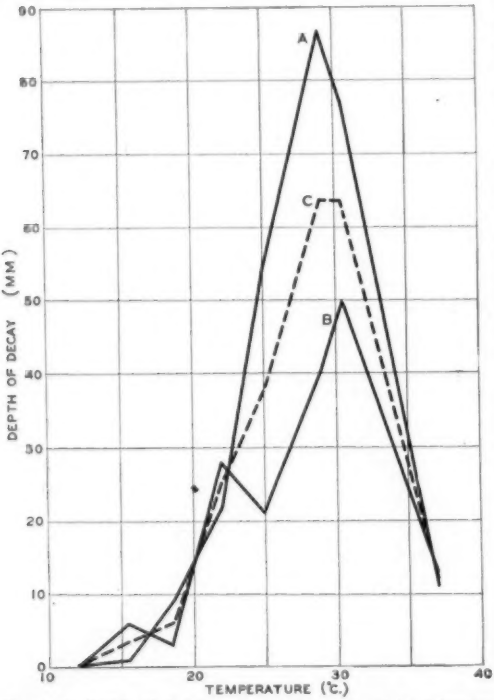

FIGURE 2.—Depth of decay in sweetpotatoes caused by *Sclerotium bataticola* at various temperatures: *A*, Roots inoculated when freshly wounded; *B*, wounded roots stored for 10 days at 13° C. and at 59 and 69 percent relative humidity before inoculation; *C*, average of *A* and *B*.

at 31°, would seem to indicate that the optimum temperature must lie between 28° and 31°.

TEMPERATURE LIMITS OF INFECTION

As has been noted in the results already discussed, the rate of infection and decay at a temperature of 12° C. is very slow. The results given in table 20 show this fact more clearly. The data were obtained from the roots stored at four relative humidities at 12° and at 15.5° and 17.5° in one of the experiments discussed in connection with table 19. The length of storage at these temperatures was 44 days, after which the roots from all the conditions of storage were stored for 16 days at 21° and 89 percent relative humidity. Not only was infection slow in taking place at all the conditions of storage but the advance of decay was relatively slight. In many instances *Sclerotium bataticola* was displaced by other organisms, especially forms of *Fusarium*. These results indicate that 12° is near the lower limit for infection and decay. Incidentally, it will be noted that the roots initially stored at 26° and 92 percent relative humidity before inoculation continued to be practically free from infection. These roots likewise remained practically free from infection by such organisms as *Fusarium*, etc., which often displace *S. bataticola* at a temperature of 12°.

TABLE 20.—*Relation of duration of storage to infection of sweetpotatoes (Yellow Jersey variety) by Sclerotium bataticola at certain temperatures and relative humidities*

Storage conditions subsequent to inoculation		Relation of indicated storage period to infection and decay in roots inoculated—																			
		Immediately following wounding								Following wounding and 10 days' storage at 13° C. and 57 percent relative humidity								Following wounding and 10 days' storage at 26° C. and 92 percent relative humidity [1]			
		12 days		20 days		44 days [2]		60 days		12 days		20 days		44 days [1]		60 days		44 days [1]		60 days	
Temperature (° C.)	Relative humidity	Infection	Depth of decay	Infection	Depth of decay	Infection	Depth of decay	Infection	Depth of decay	Infection	Depth of decay	Infection	Depth of decay	Infection	Depth of decay	Infection	Depth of decay	Infection	Depth of decay	Infection	Depth of decay
	Per cent	*Per cent*	*Mm*	*Per cent*	*Mm*	*Per cent*	*Mm*	*Per cent*	*Mm*	*Per cent*	*Mm*	*Per cent*	*Mm*	*Per cent*	*Mm*	*Per cent*	*Mm*	*Per cent*	*Mm*	*Per cent*	*Mm*
17.5	88	20	1	20	1.0	40	3.6	100	55	40	2.4	100	5.0	100	5	100	5	0	0	0	0
15.5	92	0	0	40	1.6	40	1.6	100	4.5	80	4.6	100	8.8	100	32	100	31	0	0	0	0
12.0	93	0	0	20	0.8	40	7.0	100	67	0	0	33	1.0	100	3	100	3	0	0	0	0
12.0	85	0	0	0	0	0	0			0	0	20	1.4	100	9	100	16	0	0	0	0
12.0	76	0	0	0	0	80	2.4	100	4	0	0	0	0	100	3	100	6.3	0	0	0	0
	63	0	0	0	0	100	4.6	100	7	0	0	0	0	80	2.4	100	3.8	20	------	20	------

[1] There was no infection after storage periods of 12 or 20 days.
[2] During the interval between 44 and 60 days all roots were stored at 21° C. and 89 percent relative humidity.

The upper limit for infection of sweetpotatoes by *Sclerotium bataticola* has not been accurately determined, but judged by the rate of decay at 37° C. (table 19) and by the slowness of growth at 40° and 42° (table 17), 37° approaches this limit.

EFFECT OF DURATION OF INITIAL STORAGE OF WOUNDED SWEETPOTATOES ON INFECTION BY SCLEROTIUM BATATICOLA DURING SUBSEQUENT STORAGE

In one experiment, 5 wounded roots (cross section tip) of the Yellow Jersey variety were stored at 29° C. and 96 percent relative humidity for intervals of 1 to 10 days, inclusive. At the end of 10 days all these roots, including 5 freshly wounded ones, were inoculated with *Sclerotium bataticola* by dipping the cut end into a mass of corn meal mixed with the fungus and were stored at 28° and 89 percent relative humidity for 28 days. One hundred percent of the roots inoculated when freshly wounded became infected, 80 percent of those inoculated after 1 day's storage, 40 percent of those inoculated after 2 days' storage, and 20 percent of those inoculated after 3 days' storage. None of the roots inoculated after being stored for more than 3 days became infected.

Another experiment, run with the Yellow Jersey and Nancy Hall varieties, was similar to the foregoing in all respects, except that (1) the initial and subsequent storage temperature and relative humidity were 25.5° and 94 percent, respectively, and (2) 2-day intervals between inoculations instead of 1-day intervals were used in the case of the Yellow Jersey variety. By the end of 24 days, all the Yellow Jersey roots inoculated when freshly wounded were infected and 60 percent of those stored 2 days before inoculation were infected, but none of the roots stored for more than 2 days before inoculation showed any infection. Eighty percent of the Nancy Hall roots inoculated when freshly wounded, 80 percent of those inoculated after 1 day's storage, and 100 percent of those inoculated after 2 days' storage became infected. There was no infection in any of the roots inoculated after initial storage periods longer than 2 days.

In a third experiment (table 21) cured Yellow Jersey roots were wounded in the usual fashion (cross section tip), stored at temperatures of 22°, 25°, 29°, and 31° C., respectively, combined with high humidity, for periods of 1, 2, 3, 4, and 5 days. Then they, with a check freshly wounded, were inoculated with *Sclerotium bataticola* and stored for 16 days at 25° and 94 percent relative humidity, and for an additional period of 24 days at 21° and 91 percent relative humidity.

No infection occurred in roots stored for more than 3 days at 31° C., more than 2 days at 29°, more than 2 days at 25°, or more than 4 days at 22°. Some variation in these limits of initial storage might be expected with a variation in the subsequent storage conditions. In other words, if the process of healing is not complete by the end of the initial storage period, infection may take place during subsequent storage that is sufficiently unfavorable to the healing process and sufficiently favorable to the activities of the pathogene. If the process of healing is complete by the end of the initial storage period, subsequent storage has little effect on infection.

TABLE 21.—*Effect of preliminary storage of wounded sweetpotatoes for periods of from 0 (check) to 5 days before inoculation on infection by Sclerotium bataticola during subsequent storage*

Storage conditions between time of wounding and inoculation and number of roots used				Infection and decay after inoculation and storage for periods indicated [1]			
				16 days		40 days	
Temperature (° C.)	Relative humidity	Interval	Roots used	Roots infected	Total depth of decay	Roots infected	Total depth of decay
	Percent	*Days*	*Number*	*Percent*	*Mm*	*Percent*	*Mm*
		0 (check)	10	80	47	80	336
31	94	1	5	20	10	20	18
31	94	2	5	0	0	0	0
31	94	3	5	20	3	20	8
31	94	4	5	0	0	0	0
31	94	5	5	0	0	0	0
29	97	1	5	20	5	20	3
29	97	2	5	0	0	20	4
29	97	3	5	0	0	0	0
29	97	4	5	0	0	0	0
29	97	5	5	0	0	0	0
25	95	1	5	20	3	20	3
25	95	2	5	40	23	40	34
25	95	3	5	0	0	0	0
25	95	4	5	0	0	0	0
25	95	5	5	0	0	0	0
22	93	1	5	40	91	40	145
22	93	2	5	20	5	40	33
22	93	3	5	40	95	40	182
22	93	4	5	20	4	20	3
22	93	5	5	0	0	0	0

[1] For the first 16 days storage was at 25° C. and 94 percent relative humidity; for the last 24 days at 21° and 91 percent relative humidity.

DISCUSSION AND CONCLUSIONS

Some of the factors influencing infection and decay of sweetpotatoes by the various pathogenes discussed herein are as follows: Presence or absence of the pathogene, infection and decay by other fungi, amount of inoculum, wounding—including extent, type, and degree—suberization, periderm formation, temperature, humidity, and time.

Rhizopus tritici and *R. nigricans* (rhizopus soft rot), *Fusarium oxysporum* (surface rot), and species of *Fusarium* (including *F. oxysporum*) causing end rot seem always to be associated with sweetpotatoes in storage, for in the absence of artificial inoculation some infection from these pathogenes generally takes place with certain types of wounds and under certain conditions of temperature and humidity. There are indications that *R. tritici* and *R. nigricans* are more abundantly present on the roots as the storage season advances than at harvest time. No information is available relative to the amount of *F. oxysporum* or other forms of *Fusarium* present on sweetpotatoes at different times during the storage season or the effect that a change in the amount present may have on the amount of surface rot and fusarium end rot that develops. It is probable that under certain conditions of storage there is an increase during the storage season in the amount of these fungi present in the storage

house. There are indications that *Diplodia tubericola* and *Sclerotium bataticola* are not always present on sweetpotatoes in storage. This is shown by the fact that infection rarely occurs in sweetpotatoes grown and stored in the vicinity of Washington, D. C., although they have often been stored under conditions favorable for the development of the rots produced by these pathogenes. However, these fungi are fairly common in the southern portion of the sweetpotato areas, and in some localities are associated rather consistently with sweetpotatoes in storage, and decay will develop when the handling of the roots and the conditions of storage are favorable.

Openings of some kind, such as wounds, broken rootlets, lesions produced by other fungi, etc., in the skin of sweetpotatoes, through which the pathogenes (*Rhizopus tritici*, *R. nigricans*, *Fusarium oxysporum*, other species of *Fusarium*, *Diplodia tubericola*, and *Sclerotium bataticola*) of the various diseases discussed herein may enter, seem to be an essential condition of infection in the case of each pathogene. Considerable indirect evidence obtained from time to time shows fairly conclusively that these fungi cannot penetrate the unbroken skin of the sweetpotato. This evidence is essentially that (1) infection is consistently associated with wounding and (2) that infection is consistently absent in roots in which the unbroken skin has been exposed to the action of the pathogenes for various periods in the inoculation experiments and throughout the storage season in some of the storage experiments.

Some, and probably all, of these fungi are able to penetrate sweetpotatoes through lesions produced by other fungi. The primary invader may be one of the foregoing fungi, or it may be a fungus that is able to penetrate unwounded sweetpotato tissue under special conditions (*10*). Forms of *Fusarium* have been shown to commonly invade lesions of *Mucor racemosus*, *Alternaria*, *Botrytis*, species of *Rhizopus*, and lesions of mottled necrosis. *Diplodia tubericola* and *Sclerotium bataticola* also have been observed to invade lesions produced by some of these fungi.

Injury such as that produced by freezing temperature or by a temperature of 40° C. and above may result in an invasion of some of these fungi when the roots are again restored to more normal temperatures.

In the presence of the various pathogenes, mechanical wounding is the chief predisposing condition leading to infection of sweetpotatoes in storage. The wounding chiefly responsible for decay of sweetpotatoes in storage and in transit results from harvesting and storing operations, handling in storage and in preparation for the market, gnawing by rodents in the storage house, and mishandling of railroad cars in transit. With an increase in the intensity and extent of wounding there is an increase in the percentage of infection by *Rhizopus tritici* and *R. nigricans*. The relation of degree and extent of wounding to infection by other pathogenes has not been studied. The character of the wounding is a factor in determining the occurrence of rhizopus soft rot, surface rot, and fusarium end rot. Infection by *R. tritici* and *R. nigricans* takes place more readily in bruised than in cut or in sharply broken tissues. These pathogenes rarely invade sweetpotatoes through skinned areas, and the percentage of infection is small in roots in which the tip ends have been cut or broken off.

The absence of *Rhizopus* infection in skinned areas enables *Fusarium oxysporum* to establish itself whenever storage conditions unfavorable to suberization and periderm formation are sufficiently prolonged to permit infection. Likewise the absence of *Rhizopus* infection in roots in which the tip ends are cut or broken off enables species of *Fusarium* or other organisms under certain conditions of storage to invade the wounded tissues and thus produce end rot. Invasion by species of *Fusarium* and *Penicillium* occurs in wounded tissues of any kind when the conditions of storage immediately after wounding are unfavorable for healing, the duration of storage is sufficiently long, and the wounds are not invaded by species of *Rhizopus*, *Diplodia tubericola*, or *Sclerotium bataticola*.

The presence of a suberized layer in wounded tissue at times seems to inhibit infection by various pathogenes, and in some instances may prevent infection, although this fact has not been clearly established. Since the formation of a suberized layer is an essential preliminary to periderm formation, its power of inhibition in some instances probably is a decisive factor in delaying infection until the more effective barrier (the periderm) is developed. A periderm coextensive with the wounded area forms an almost, if not quite, perfect barrier against infection by any of the foregoing pathogenes.

Infection of wounded sweetpotatoes by the various pathogenes sometimes occurs during storage subsequent to a 10 days' initial storage at temperatures of 12° to 19° C. combined with high humidity. Suberization occurs at all these temperatures (12° to 19°) within 10 days. The presence of such infection during subsequent storage indicates that either suberization is not coextensive with the wounded tissue by the end of the initial 10-day storage or that it is not an absolute barrier to infection. The earliest that periderm formation has been observed to occur at 12° is 25 days; in one instance it occurred within 11 days at 14.7°, but in another it was absent after 10 days at 15.7°, and 11 days was required for its formation at 19°. The time required for periderm formation also varied somewhat with the lot of roots used, and even if it was found present in a given case it may not always have been coextensive with the wounds. The histological study was confined to limited samples that were representative of the tissues involved but were never coextensive with the wounded tissues concerned in the infection experiments. It will be seen from these considerations that 10-day storage at temperatures of 12° to 19° probably does not always insure complete healing. In the absence of healing during the initial storage period some infection may occur or periderm formation may go on to completion, depending upon the temperature and humidity of the subsequent storage.

Temperature, in addition to being an indirect factor in infection and decay of sweetpotatoes because of its influence on suberization and periderm formation, is a direct factor in determining the limits of infection and in its effect on the rate of infection and decay.

That either free moisture or high atmospheric humidity is favorable to the growth and infection activities of the various pathogenes is shown by the fact that they grow well on carrot agar and readily decay the moist tissue of sweetpotatoes. It is believed that air moisture may become a limiting factor in the growth and infection activities of these fungi if reduced beyond a certain atmospheric content. This problem has not been the object of special study except in the case of infection by *Rhizopus* under certain conditions.

At a temperature of 23° C. infection in halved roots is limited at relative humidities below 75 percent and almost excluded at relative humidities of 48 to 50 percent, because of the effect of this reduction in moisture upon the pathogenes. The effects of relative humidity on these fungi at temperatures of 12° and 28° are not so definitely known. Atmospheric humidity is a more important factor as it indirectly affects infection by all the storage rot fungi under consideration through its effect on suberization and periderm formation than as it affects these fungi directly. Not only is this healing process limited to certain relative humidities, but its rate is increased as the relative humidity is raised above the lower limit of suberization and periderm formation.

The time required for the initiation of decay by the various pathogenes varies with the pathogene, the temperature, and the humidity of the storage chamber. This period also may be affected by the rate of suberization and periderm formation, which also varies with the temperature and humidity of the storage chamber. Whether the period during which wounded sweetpotato roots are exposed to a given storage condition is a limiting factor in infection depends on its duration and on the disease, the amount of inoculum present, the type of wounding, and the subsequent storage conditions.

SUMMARY

During storage the infection of sweetpotatoes by the various storage-rot fungi is influenced by the occurrence of these pathogenes. *Rhizopus nigricans*, *R. tritici*, *Fusarium oxysporum*, and species of *Fusarium* (including *F. oxysporum*) that cause end rot are generally found associated with sweetpotatoes in storage, whereas the occurrence of *Diplodia tubericola* and *Sclerotium bataticola* is more regional, being more commonly limited to the southern portion of the sweetpotato area.

Injury of some kind, such as is produced by wounds, by extremes of temperature, or by other fungi, seems to be an essential preliminary to infection by all the foregoing pathogenes. Mechanical wounding is by far the most important type of injury leading to infection.

Infection of sweetpotatoes by the various pathogenes is influenced by the extent, degree, and kind of wounding. *Rhizopus* infection is favored by bruising and extended wounding, and rarely occurs in skinned areas or in roots with tip ends sharply broken or cut off. Surface rot (*Fusarium oxysporum*) occurs in skinned areas, through injured sprouts or in tissue beneath the sprouts that has been wounded by breaking them off, and through broken secondary rootlets in uncured roots. Species of *Fusarium* invade wounded tissues in roots stored at conditions of temperature and humidity that prevent or greatly retard wound-cork formation. A large percentage of the roots with tip ends cut or broken off and stored under such conditions become infected with *Fusarium*.

A suberin layer seems to inhibit and may sometimes prevent infection by the various fungi.

A wound-periderm layer is an effective barrier against infection by various storage fungi and retards the loss of moisture and consequent shriveling.

Judged from the infection data as well as the histological study, the rate of wound healing tends to increase as the temperature (combined with relative humidities above 90 percent) is raised from 12° to

about 32° C., when it commences to decline. Twenty-five days was required for periderm formation at 12°.

If the moisture content of the air of the storage chamber is reduced beyond a particular point at each of the temperatures 12°, 23°, and 28° to 29° C., healing of wounds in sweetpotatoes will not occur. As the moisture content of the air is raised above this point the rate of healing tends to increase. The range of relative humidities at 12° at which healing occurs is narrow (between 90 and 100 percent). As the temperature is raised to 23° and 28° to 29° the range of relative humidities at which healing takes place becomes progressively wider, the extent of the range depending somewhat on the duration of storage.

Temperature as it affects the fungi producing the various rots is a factor in determining the rate and limits of infection. Although rate of infection increases as the temperature is raised above a minimum, the data show that the increase in rate is not as marked as in the case of wound healing.

There are indications in certain instances that as the moisture content of the air of the storage room is reduced beyond a certain point it tends to limit infection by its action on the pathogenes.

The optimum, minimum, and maximum temperatures for growth of *Diplodia tubericola* are approximately 29° to 31°, 8° to 13°, and 37° to 40° C., respectively. Infection of sweetpotatoes by *D. tubericola* has been obtained at temperatures from 12° to 37°, the most rapid decay occurring at 29° to 31°. The optimum, minimum, and maximum temperatures for growth of *Sclerotium bataticola* are approximately 31°, 8°, and 42° C., respectively. Infection of sweetpotatoes by *S. bataticola* has been obtained at temperatures from 12° to 37°, the most rapid decay occurring at 29° to 31°.

The data presented show that if sweetpotatoes are subjected to 10 days of curing at temperatures from 22° to 32° C., combined with relative humidities of 90 percent and above, protection is developed in wounded tissue against the loss of moisture and consequent shriveling and against infection by *Rhizopus tritici*, *R. nigricans*, *Fusarium oxysporum*, species of *Fusarium* causing end rot, *Diplodia tubericola*, and *Sclerotium bataticola*. These and other data show that this protection is more effectively maintained in storage at 10° to 15° C. if the relative humidity, after curing, is above 80 percent.

If wounded sweetpotatoes are stored 10 days at temperatures from 12° to 37° C., combined with relative humidities above 90 percent, before inoculation in the case of *Diplodia tubericola* and *Sclerotium bataticola* and without inoculation in the case of *Rhizopus tritici*, *R. nigricans*, *Fusarium oxysporum*, and species of *Fusarium* causing end rot, infection during subsequent storage at different conditions is usually limited to roots stored at initial temperatures of 12° to 19° C. (sometimes as high as 21°), increasing with a lowering of the temperature. Since storage for 10 days at initial temperatures between 21° and 32° (combined with high humidity) permitted the wounded tissue to heal, these combinations gave almost complete protection against infection during subsequent storage at various conditions of temperature and relative humidity. As the initial temperatures were raised above 32° there was sometimes greater infection during subsequent storage than in roots initially stored at 21° to 32°.

Five days of initial storage of wounded sweetpotatoes at 25° C. and 94 percent relative humidity and 3 days at 29° and 96 percent

relative humidity, before inoculation, were required for the roots to develop sufficient wound periderm to prevent infection by *Diplodia tubericola* during subsequent storage. In the case of *Sclerotium bataticola*, the length of initial storage required to prevent infection during subsequent storage was 4 days at 31° and 94 percent relative humidity, and at 29° and 96 to 97 percent relative humidity, 3 days at 25° and 95 percent relative humidity, and 5 days at 22° and 94 percent relative humidity.

Infection by *Diplodia tubericola* and *Sclerotium bataticola* took place as readily in roots wounded and stored for 10 days at 13° C. and relative humidities of 59 and 69 percent before inoculation as in roots inoculated when freshly wounded, showing that healing had not taken place under these conditions. A 10-day initial storage of wounded roots, before inoculation, at 26° and 29°, combined with 92 and 97 percent relative humidity, respectively, prevented infection during subsequent storage, showing that these initial storage conditions were favorable to wound healing.

LITERATURE CITED

(1) Artschwager, E., and Starrett, R. C.
1931. suberization and wound-periderm formation in sweetpotato and gladiolus as affected by temperature and relative humidity. Jour. Agr. Research 43: 353–364, illus.

(2) Harter, L. L., Lauritzen, J. I., and Weimer, J. L.
1923. mottle-necrosis of sweet potatoes. Phytopathology 13: 145–146, illus.

(3) ——— and Weimer, J. L.
1921. studies in the physiology of parasitism with special reference to the secretion of pectinase by rhizopus tritici. Jour. Agr. Research 21: 609–625.

(4) ——— and Weimer, J. L.
1921. a comparison of the pectinase produced by different species of rhizopus. Jour. Agr. Research 22: 371–377, illus.

(5) ——— and Weimer, J. L.
1923. the relation of the enzym pectinase to infection of sweet potatoes by rhizopus. Amer. Jour. Bot. 10: 245–258.

(6) ——— Weimer, J. L., and Adams, J. M. R.
1918. sweet-potato storage-rots. Jour. Agr. Research 15: 337–368, illus.

(7) Hauman-Merck, L.
1913. contribution à l'étude des altérations microbiennes des organes charnus des plantes. Ann. Inst. Pasteur 27: 501–522.

(8) Lauritzen, J. I.
1926. infection and temperature relations of black rot of sweet potatoes in storage. Jour. Agr. Research 33: 663–676, illus.

(9) ———
1926. a strain of yellow jersey sweet potato resistant to surface rot (fusarium oxysporum schlecht). Jour. Agr. Research 33: 1091–1094.

(10) ———
1931. some effects of chilling temperatures on sweetpotatoes. Jour. Agr. Research 42: 617–627, illus.

(11) ——— and Harter, L. L.
1923. species of rhizopus responsible for the decay of sweet potatoes in the storage house and at different temperatures in infection chambers. Jour. Agr. Research 24: 441–456, illus.

(12) ——— and Harter, L. L.
1926. the relation of humidity to infection of the sweet potato by rhizopus. Jour. Agr. Research 33: 527–539, illus.

(13) Weimer, J. L., and Harter, L. L.
1921. wound-cork formation in the sweet potato. Jour. Agr. Research 21: 637–647.

VARIATION AND CORRELATION IN GRAIN YIELD AMONG 1,500 WHEAT NURSERY PLOTS[1]

By GUSTAV A. WIEBE[2]

Assistant agronomist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

In the future, increased grain yields of wheat probably will be secured by smaller and smaller increments, and the agronomist will have to use a finer, more accurate "sieve" in order to isolate the more productive wheats. This paper analyzes the variation and correlation in grain yield among 1,500 wheat nursery plots. Certain of the facts outlined will, the writer believes, permit the estimation of a smaller experimental error and show why some seeding arrangements give better results than others.

In the past many experiments have been conducted to study the variability of field crops of all kinds and the means of reducing it to a minimum. For this purpose uniformity trials similar to the one about to be considered have generally been used. Comparatively little work has been done, however, toward determining the correlation between adjacent plots and those at a greater distance apart. This paper, therefore, is largely concerned with this part of the problem in relation to the general question of variability. The following investigators have considered various phases of the question: Mercer and Hall (*15*),[3] Hall and Russell (*9*), Montgomery (*16, 17*), Kiesselbach (*13*), Lyon (*14*), Day (*3*), Stadler (*18, 19*), Hayes (*12*), Forster and Vasey (*6*), Garber, McIlvaine, and Hoover (*7*), Stringfield (*20*), Student (*1*), Summerby (*21*), Wood and Stratton (*24*), Griffee (*8*), Engledow and Yule (*4*), Christidis (*2*), and Harris (*10, 11*). These are referred to later as they touch upon the subject matter considered.

MATERIAL AND METHODS

The material for this study consisted of 1,500 rows of Federation wheat (C. I. no. 4734),[4] which is well adapted to the region where it was grown. The seed was pure. The crop was grown in the summer of 1927 on the west end of series 100 on the Aberdeen Substation, Aberdeen, Idaho. The ground had been uniformly cropped the previous year to field peas. The plot was seeded on April 18 with a grain drill that sowed eight rows at a time. The rows were 12 inches apart and 15 feet long. Because the land sloped gently to the south

[1] Received for publication Sept. 29, 1934; issued April 1935. This paper is based on cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Idaho and California Agricultural Experiment Stations. Submitted to the faculty of the University of California in partial fulfillment of the requirements for the degree of doctor of philosophy.

[2] The writer acknowledges valuable suggestions from Dr. S. C. Salmon, principal agronomist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and from Dr. R. E. Clausen, Division of Genetics, Dr. F. N. Briggs, Division of Agronomy, Prof. C. M. Titus, Division of Mathematics, and Prof. E. R. Parker, Citrus Experiment Station, Riverside, Calif., of the University of California.

[3] Reference is made by number (italic) to Literature Cited, p. 356.

[4] Accession number of the Division of Cereal Crops and Diseases.

and west, levees were necessary at 15- and 30-foot intervals. Figure 1 shows the general arrangement and the system of identifying individual series and rows. During the growing season the crop was

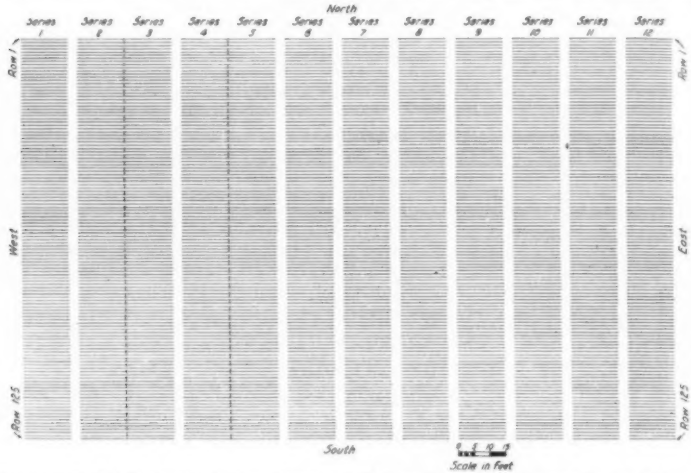

FIGURE 1.—Field plan of uniformity experiments, showing general arrangement of rows. Series 2 and 3, 4 and 5, were grown as 30-foot rows but were cut in 15-foot lengths at harvest time.

irrigated four times. The average annual rainfall at Aberdeen is about 7 inches. The individual rows were harvested in August and

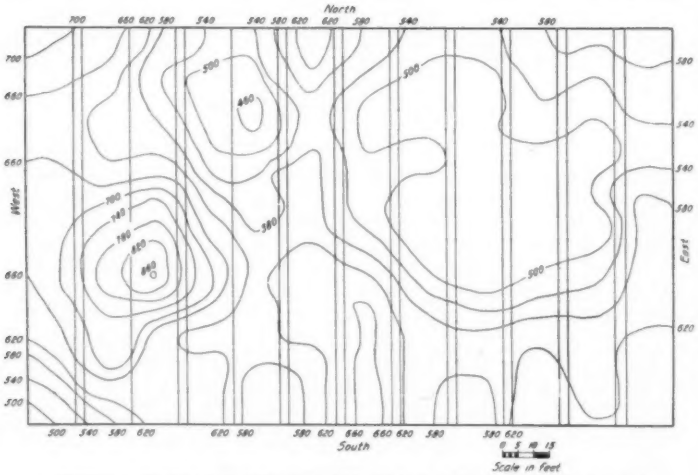

FIGURE 2.—Contour map of the grain yields obtained. The lines are at 40-g intervals.

threshed with a small nursery thresher. The grain yields, recorded in grams per row, are shown in table 1. A contour map of the yields obtained in the plot is shown in figure 2.

The wheat yielded 63 bushels per acre, which is above the average for that region. No disease or insect damage was noticed. Although the height of plants and the weight of straw per row were recorded, only the yield of grain is discussed in this paper.

TABLE 1.—*Yield of grain from each of 1,500 15-foot rows of wheat, 12 inches apart, grouped in 12 series of 125 rows each*

Series 1	Series 2	Series 3	Series 4	Series 5	Series 6	Series 7	Series 8	Series 9	Series 10	Series 11	Series 12
715	595	580	580	615	610	540	515	557	665	560	612
770	710	655	675	700	690	565	585	550	574	511	618
760	715	690	690	655	725	665	640	665	705	644	705
665	615	685	555	585	630	550	520	553	616	573	570
755	739	670	580	545	620	580	525	495	565	599	612
745	670	585	560	550	710	590	545	538	587	600	664
645	690	550	520	450	630	535	505	530	536	611	578
585	495	455	470	445	555	500	450	420	461	531	559
560	540	450	500	505	595	545	530	498	538	453	600
685	730	610	500	555	645	605	535	534	593	616	638
755	810	665	570	525	715	650	550	613	607	742	657
640	635	585	465	430	615	550	515	369	493	635	567
725	655	530	455	465	560	550	460	455	503	519	555
715	775	615	545	530	685	595	510	507	561	581	537
700	705	555	440	455	630	555	425	476	648	532	552
640	655	495	435	485	585	475	495	422	516	458	559
620	635	495	445	455	621	505	465	419	591	545	537
750	630	555	455	510	575	530	470	427	545	562	582
760	675	610	540	530	675	580	460	513	599	595	675
630	645	490	445	440	585	490	420	460	542	474	563
625	540	495	415	445	530	440	430	455	485	461	533
670	670	560	485	445	590	555	459	516	570	533	623
655	630	540	465	460	560	465	435	466	454	458	503
570	535	525	455	415	510	460	420	393	482	423	446
575	495	540	480	395	555	440	459	437	541	525	514
690	645	595	515	470	550	515	445	508	508	517	533
730	730	710	565	465	640	565	550	494	591	594	597
620	635	595	485	435	530	490	470	465	521	454	537
620	600	530	455	425	545	395	455	482	484	505	578
665	685	685	520	480	525	550	465	497	596	503	634
620	605	530	440	450	500	470	445	510	531	478	514
615	525	610	515	395	510	455	530	485	554	448	503
595	625	600	440	435	510	515	525	500	522	495	514
640	580	630	485	480	610	525	515	574	525	458	518
695	650	695	550	515	685	570	525	613	560	532	525
630	610	545	465	460	555	500	575	438	477	480	507
595	555	580	505	465	540	425	490	467	479	444	507
645	635	660	550	525	645	515	520	477	510	465	533
705	660	595	495	465	595	450	505	417	489	359	533
575	530	600	460	410	500	460	505	459	431	438	578
530	555	570	450	425	490	450	495	426	443	449	499
715	680	685	505	535	600	495	480	474	491	495	533
795	715	740	590	585	585	535	560	518	513	541	548
660	635	620	495	510	585	520	475	444	495	509	499
615	580	595	490	505	555	450	470	468	442	474	484
690	645	630	600	565	645	530	550	454	474	502	548
675	615	640	505	550	615	535	500	465	456	513	563
770	625	685	570	575	685	540	475	534	456	467	548
695	700	770	540	515	685	530	495	508	454	422	600
690	660	705	570	545	645	500	540	494	500	532	687
720	730	825	665	605	665	520	550	584	546	537	630
670	640	710	500	535	575	495	455	512	497	465	600
655	590	675	575	515	570	490	470	460	427	472	589
780	790	855	625	575	655	530	540	575	502	503	582
750	705	770	575	560	565	510	435	486	470	502	570
585	620	685	620	550	520	435	490	429	485	534	619
665	605	680	570	560	560	445	495	461	463	533	627
735	760	790	645	605	595	560	485	466	487	476	645
795	795	850	700	655	635	540	470	521	582	584	759
645	685	720	610	520	550	460	440	425	485	462	698
630	695	665	630	510	575	445	430	433	423	450	627
685	840	875	640	575	625	570	490	514	539	486	717
695	780	790	575	520	690	545	470	455	486	488	619
765	745	840	710	640	610	590	530	498	479	434	574
655	735	840	640	610	585	560	459	469	562	450	570
640	880	795	660	580	615	585	460	461	472	504	600
790	765	890	770	660	690	575	490	445	475	509	582
670	690	785	645	510	560	535	495	474	455	458	555
685	790	770	665	600	545	555	390	389	451	439	615
660	825	960	750	660	680	620	510	501	490	534	694
705	805	860	635	540	650	555	435	479	457	451	649
610	720	705	615	540	625	595	380	379	483	502	608
640	735	805	665	535	605	580	430	427	441	498	638
690	855	905	700	615	650	615	495	531	448	453	645
715	765	945	820	695	750	685	530	511	580	510	762

TABLE 1.—*Yield of grain from each of 1,500 15-foot rows of wheat, 12 inches apart, grouped in 12 series of 125 rows each*—Continued

Series 1	Series 2	Series 3	Series 4	Series 5	Series 6	Series 7	Series 8	Series 9	Series 10	Series 11	Series 12
685	750	825	715	580	660	655	505	414	451	542	642
685	790	790	695	595	625	510	525	425	471	478	514
775	845	995	829	655	675	649	545	474	513	599	597
645	870	890	749	615	655	555	490	433	558	590	567
725	825	920	649	600	690	575	535	451	484	510	567
650	805	935	690	645	640	530	520	476	507	525	563
695	860	960	725	615	710	660	525	438	539	646	623
735	910	975	775	680	700	770	635	506	528	514	638
645	745	815	700	695	615	605	550	455	504	503	589
630	810	730	635	535	650	735	535	458	554	535	615
680	745	840	730	645	650	775	590	557	561	565	619
620	730	775	680	610	610	680	515	537	535	553	570
620	745	660	565	520	635	610	450	476	559	562	575
560	675	690	635	525	605	635	515	494	516	558	559
625	700	725	645	645	580	615	640	516	618	615	604
700	765	725	615	640	705	710	590	619	627	620	645
685	785	655	690	570	615	670	575	506	602	530	600
625	550	590	590	605	505	560	595	539	629	571	698
745	790	675	600	625	685	725	695	686	608	568	672
680	670	630	640	645	650	695	610	627	579	537	675
655	730	615	650	640	645	655	620	611	642	685	630
625	700	675	720	695	680	720	615	595	556	648	578
670	735	645	620	705	695	650	580	623	541	580	630
670	820	685	665	715	715	790	660	658	689	725	675
555	670	590	580	550	665	640	605	553	580	605	638
650	685	505	525	595	590	560	575	592	559	584	518
625	760	625	545	635	635	670	625	672	656	577	645
629	740	575	565	595	610	650	595	515	641	533	623
580	650	570	575	450	445	670	645	587	634	534	574
615	705	550	515	530	485	610	570	565	652	518	623
675	690	615	610	560	625	745	540	584	616	595	668
720	820	720	650	590	615	770	645	615	755	625	713
640	780	640	660	605	540	695	580	615	679	540	570
630	800	715	710	605	575	490	485	564	665	665	582
625	830	795	765	610	630	775	630	614	760	730	619
470	685	680	735	560	580	670	610	556	539	488	608
445	555	605	685	540	615	610	525	640	639	594	739
400	570	555	655	475	615	715	595	595	740	671	795
480	495	650	690	535	600	760	655	488	621	578	615
555	660	715	690	635	655	775	665	652	720	689	728
520	530	650	605	480	545	585	610	561	670	622	623
490	475	595	660	475	545	635	550	524	538	626	552
500	610	590	680	555	625	670	660	581	613	583	585
425	560	570	570	455	605	605	530	544	645	612	537
535	550	540	630	480	565	745	515	467	606	563	510
560	500	560	575	500	475	610	575	492	591	532	499
530	610	600	620	480	585	650	570	577	612	654	630
570	585	635	765	550	675	765	620	608	705	677	660
505	500	580	655	470	595	570	555	537	585	589	619
465	430	510	680	460	600	670	615	620	594	616	784

Five aspects of variation and correlation as a general problem are considered here: (1) Tne general variability of the field, considered under an extended and a closed system; (2) the correlation between rows at different distances apart; (3) the intraclass correlation and variation within combination plots compounded by different systems of replication; (4) the variation between combination plots compounded by different systems of replication; (5) the principle of maximum contiguity and its practical application.

EXPERIMENTAL RESULTS

GENERAL VARIABILITY UNDER AN EXTENDED AND A CLOSED SYSTEM

In discussing general variability in yield, one must clearly distinguish between the extended system and the closed system.

In the present discussion an extended system is one in which, at each successive consideration of variability, more or less land is used. For example, if the variation of the 125 rows in series 1 is found, and the 125 rows of series 2 are added, making a total of 250 rows (each 15 feet long), and the variation is again computed, then the general variability is investigated under an extended system. One may

increase or decrease the number of units of the same size, or one may keep their number constant and vary their size. In the present case both systems were used but always going from a smaller area of land to a larger one.

In the closed system, on the other hand, the area of land remains the same, but the size and number of plots change. Under this system the variability of numerous smaller plots is studied first, and then a few larger plots are considered. In this study the increase in plot size may conveniently be limited to an increase in length of row. The size might also be increased by adding together rows lying side by side. The effect of shape of plot is discussed later.

The problems to be determined in such a study of plot variability are: (1) The variation when more and more land is added to the experimental area while the size and shape of the ultimate plot remain the same; (2) the effect when more and more land is added and the number of plots remains the same but their size is automatically increased; and (3) the variation when the entire area is used at each consideration but the plots differ in size.

Many studies have already dealt with plot variability, but the literature does not often state under which of the two systems the work was done.

To determine the general variability under the systems outlined above, the mean, the standard deviation, and the coefficient of variation were calculated for each of the 12 series. These figures are given in table 2. The coefficients of variation ranged from 9.98 for series 6 to 18.14 for series 3. The contour map (fig. 2) shows why this is true. Series 3 includes an area of relatively high yields, such as series 6 does not contain. More contour lines cross series 3 than series 6, and this map roughly indicates the variability existing at different points.

TABLE 2.—*Mean, standard deviation, and coefficient of variation of grain yield for individual series of nursery plots of wheat*

[Each series included 125 rows 15 feet in length]

Series no.	Mean yield per row	Standard deviation	Coefficient of variation
	Grams	*Grams*	
1	644.58±[1]7.29	81.47±[1]5.15	12.64±[1]0.81
2	682.98± 8.96	100.23± 6.34	14.68± .95
3	673.38±10.93	122.18± 7.73	18.14± 1.18
4	596.26± 8.51	95.18± 6.02	15.96± 1.03
5	544.74± 6.92	77.42± 4.90	14.21± .92
6	609.38± 5.44	60.84± 3.85	9.98± .64
7	581.22± 8.28	92.57± 5.85	15.93± 1.03
8	524.26± 6.11	68.32± 4.32	13.03± .84
9	510.18± 6.21	69.46± 4.39	13.62± .88
10	546.66± 6.83	76.34± 4.83	13.96± .90
11	541.22± 6.48	72.40± 4.58	13.38± .86
12	599.78± 6.07	67.91± 4.30	11.32± .73

[1] Standard error.

Table 3 gives the mean, standard deviation, and coefficient of variation, as determined under the extended system, where the plot size remains the same but successive increments of land are added. The upper section of table 3 shows the variation existing among the 125 rows of series 1 at the west side of the field and then the change in variation from west to east as the other series (125 rows each) are added one by one. In the lower section of table 3 the same method is followed, from east to west. The coefficients of variation so deter-

mined are shown graphically in figure 3. As indicated by the data, variability tends to increase as more land is brought into the experimental area. Although the lines in figure 3 fluctuate somewhat, the trend is upward. As such increased variability is not expected if the laws of random sampling are fulfilled, evidently the laws of sampling have not been strictly adhered to. The difficulty lies in the correlation existing between nearby rows and the significant differences between most of the means and standard deviations of the 125 rows of the 12 series under investigation. The trend, therefore, should be upward, as is shown by table 2 in conjunction with table 3. To illustrate this point, consider series 1 and 2. Series 1 has a mean of 644.58 g and a coefficient of variation of 12.64 percent. Now, if series 2, which has a mean of 682.98 g and a coefficient of variation of 14.68 percent, is grouped with series 1, the inevitable result is increased variability for the larger area. Changes in variability of increased areas depend on two factors, namely, the mean and variability of the rows to be added in relation to the mean and variability of the rows already in the experiment. Reduced variability of the enlarged experimental area is expected if the rows to be added show less variability than those already under consideration and have a mean reasonably close to the latter, and also if they have an extremely small variability and a mean considerably different from that already in the experiment. On the other hand, increased variability is expected if the rows to be added have a greater variability than those already in the experiment and if their mean is reasonably close to the latter, and also if the rows to be added have less variability than those already in the experiment but a mean markedly different from the latter. Sometimes the mean and sometimes the variability of the areas added is the more important factor in changing the variability of the larger area.

TABLE 3.—*Mean, standard deviation, and coefficient of variation of grain yield for different series groupings, from west to east and from east to west*

WEST TO EAST

Series grouped (nos.)	Rows [1]	Mean yield per row	Standard deviation	Coefficient of variation
	Number	*Grams*	*Grams*	
1	125	644.58±[2]7.29	81.47±[2]5.15	12.64±[2]0.81
1, 2	250	663.78± 5.90	93.33± 4.17	14.06± .64
1, 2, 3	375	666.98± 5.37	103.94± 3.80	15.58± .58
1, 2, 3, 4	500	649.30± 4.76	106.33± 3.36	16.38± .53
1, 2, 3, 4, 5	625	628.39± 4.38	109.51± 3.10	17.43± .51
1, 2, 3, 4, 5, 6	750	625.22± 3.77	103.25± 2.67	16.51± .44
1, 2, 3, 4, 5, 6, 7	875	618.94± 3.48	102.94± 2.46	16.63± .41
1, 2, 3, 4, 5, 6, 7, 8	1,000	607.10± 3.29	104.10± 2.33	17.15± .39
1, 2, 3, 4, 5, 6, 7, 8, 9	1,125	596.33± 3.14	105.34± 2.22	17.66± .38
1, 2, 3, 4, 5, 6, 7, 8, 9, 10	1,250	591.36± 2.94	103.89± 2.08	17.57± .35
1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11	1,375	586.80± 2.76	102.45± 1.95	17.46± .34
1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12	1,500	587.89± 2.58	100.09± 1.83	17.03± .32

EAST TO WEST

12	125	599.78±[2]6.07	67.91±[2]4.30	11.32±[2]0.73
12, 11	250	570.50± 4.81	76.05± 3.40	13.33± .61
12, 11, 10	375	562.55± 3.97	76.97± 2.81	13.68± .51
12, 11, 10, 9	500	549.46± 3.51	78.51± 2.48	14.29± .46
12, 11, 10, 9, 8	625	544.42± 3.09	77.24± 2.18	14.19± .41
12, 11, 10, 9, 8, 7	750	550.55± 2.96	81.17± 2.10	14.74± .39
12, 11, 10, 9, 8, 7, 6	875	558.96± 2.75	81.24± 1.94	14.53± .35
12, 11, 10, 9, 8, 7, 6, 5	1,000	557.18± 2.56	80.90± 1.81	14.52± .33
12, 11, 10, 9, 8, 7, 6, 5, 4	1,125	561.52± 2.49	83.52± 1.76	14.87± .32
12, 11, 10, 9, 8, 7, 6, 5, 4, 3	1,250	572.71± 2.67	94.32± 1.89	16.47± .34
12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2	1,375	582.73± 2.70	100.04± 1.91	17.17± .34
12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1	1,500	587.89± 2.58	100.09± 1.83	17.03± .32

[1] Each row was 15 feet in length. [2] Standard error.

In table 4, under the extended system of determining general variability, the number of plots is kept constant but their size is increased by lengthening the rows, or, in other words, by adding together the

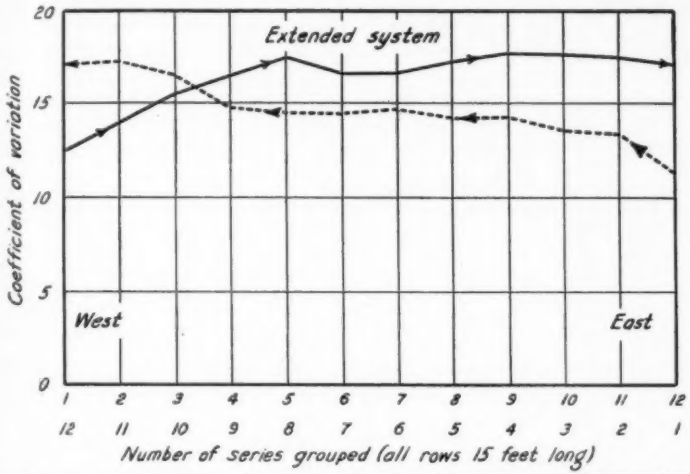

FIGURE 3.—Trend of the coefficient of variation, under an extended system, when different numbers of series are grouped, from west to east (solid line) and from east to west (dotted line).

yields from rows the ends of which were adjacent to each other. The general trend of the coefficient of variation now is downward (fig. 4). In the former extended system, where the size of plot was

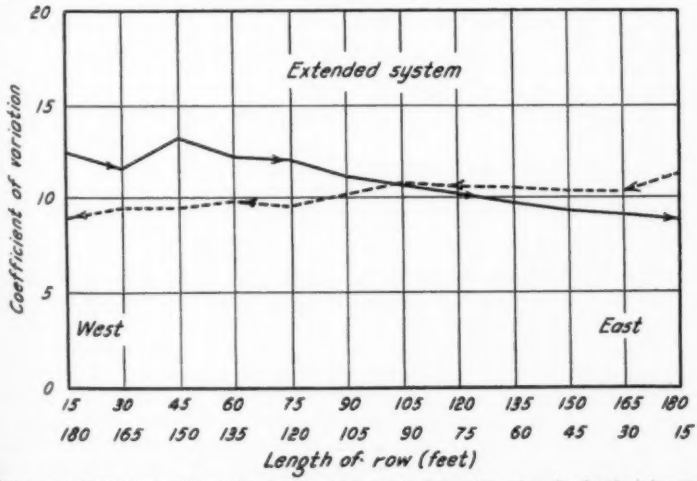

FIGURE 4.—Trend of the coefficient of variation, under an extended system, when the plot size is increased by lengthening the row, from west to east (solid line) and from east to west (dotted line).

kept constant, the variability tended to increase; but in the present case, where the plots are allowed to increase in size while remaining the same in number, variability tends to decrease. This reduction

may be explained on the basis that when the rows are added—for example, row 1 in series 1 and row 1 in series 2—the positive and the negative deviations tend to cancel each other. The variability is not reduced in proportion to the square root of the number of rows combined. It is less than expected, because correlation exists between the rows combined. The sharp increase in the coefficient of variation in table 4 when series 3 was added undoubtedly is a result of the high variability of this series, the coefficient of variation being 18.14 percent (table 2). In most instances the averages of the rows added together were more variable than those of the rows already in the experiment.

TABLE 4.—*Mean, standard deviation, and coefficient of variation of grain yield for different series groupings, of 125 rows of various lengths, from west to east and from east to west* [1]

WEST TO EAST

Series grouped (nos.)	Length of row	Mean yield per row	Standard deviation	Coefficient of variation
	Feet	*Grams*	*Grams*	
1	15	644.58±[2]7.29	81.47±[2]5.15	12.64±[2]0.81
1, 2	30	1,332.10±14.00	156.57± 9.90	11.75± .75
1, 2, 3	45	2,002.66±23.50	262.70±16.61	13.12± .84
1, 2, 3, 4	60	2,600.42±28.58	319.55±20.21	12.29± .79
1, 2, 3, 4, 5	75	3,144.74±34.05	380.69±24.08	12.11± .78
1, 2, 3, 4, 5, 6	90	3,750.50±37.06	414.30±26.20	11.05± .71
1, 2, 3, 4, 5, 6, 7	105	4,372.26±42.34	473.33±29.94	10.83± .69
1, 2, 3, 4, 5, 6, 7, 8	120	4,848.74±44.44	496.80±31.42	10.25± .66
1, 2, 3, 4, 5, 6, 7, 8, 9	135	5,369.06±46.81	523.39±33.10	9.75± .62
1, 2, 3, 4, 5, 6, 7, 8, 9, 10	150	5,910.50±49.82	557.04±35.23	9.42± .60
1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11	165	6,457.70±53.02	592.79±37.49	9.18± .59
1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12	180	7,054.50±56.76	634.56±40.13	9.00± .57

EAST TO WEST

12	15	599.78±[2]6.07	67.91±[2]4.30	11.32±[2]0.73
12, 11	30	1,134.98±10.55	117.97± 7.46	10.39± .66
12, 11, 10	45	1,682.98±15.68	175.32±11.09	10.42± .67
12, 11, 10, 9	60	2,196.26±20.73	231.82±14.66	10.56± .68
12, 11, 10, 9, 8	75	2,721.38±25.65	286.74±18.14	10.54± .67
12, 11, 10, 9, 8, 7	90	3,303.14±32.20	360.02±22.77	10.90± .70
12, 11, 10, 9, 8, 7, 6	105	3,918,50±35.45	396.34±25.07	10.11± .65
12, 11, 10, 9, 8, 7, 6, 5	120	4,458.02±38.54	430.91±27.25	9.67± .62
12, 11, 10, 9, 8, 7, 6, 5, 4	135	5,057.06±44.94	502.42±31.78	9.93± .63
12, 11, 10, 9, 8, 7, 6, 5, 4, 3	150	5,737.70±49.40	552.35±34.93	9.63± .61
12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2	165	6,405.82±54.72	611.76±38.69	9.55± .61
12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1	180	7,054.50±56.76	634.56±40.13	9.00± .57

[1] The rows were lengthened by adding together the yields from rows which had their ends adjacent to each other.
[2] Standard error.

Under the closed system, a constant area of land is considered but different-sized subunits are used in studying its variability. In each study, furthermore, all the land is used. Table 5 records the data from a treatment of this kind. The size and number of the plots must necessarily change as the study progresses. The change in size in these data has been limited to a lengthening of rows. Figure 5 shows graphically the relationships found. Under the closed system, theoretical curves can be calculated and compared with the actual one. These curves also are shown in figure 5. Only certain row lengths can utilize all the land each time the variability is determined. These lengths are 15, 30, 45, 60, 90, and 180 feet. A gradual reduction in variability occurs as the length of row is increased. For the

15-foot rows it is 17.03 percent, for the 180-foot rows 9.00 percent, of the mean yield. Since increasing the size of plot is equivalent to averaging adjacent plots, this reduction in variability should be proportional to the square root of the number of plots in each group when, and only when, the intraclass correlation coefficient between the plots grouped is zero. The theoretical curve of reduction in variability under these conditions is the lowest one shown in figure 5. It certainly does not agree with the actual results.

Since adjacent rows are positively correlated, as will be shown later, it is interesting to see what form the theoretical curve takes in comparison with the actual when this fact is considered. To compute the

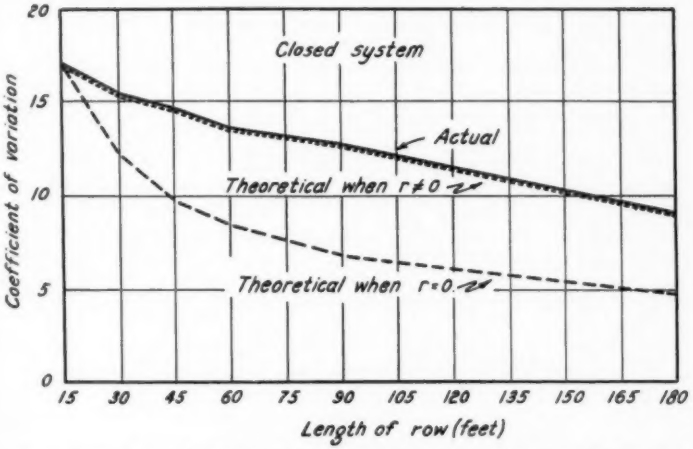

FIGURE 5.—Trend of the coefficient of variation, under a closed system, when rows of different lengths are considered. The theoretical lines when $r=0$ and $r\neq 0$ are also shown.

variability with r as an influencing factor, the following formula given by Yule (*25, p. 350*) was used:

$$\sigma_m^2 = \frac{\sigma_p^2}{n}[1+(n-1)r]$$

where σ_m is the standard deviation of the combination plots desired, σ_p the standard deviation of the ultimate plots, n the number of ultimate plots in the combination plot, and r the intraclass coefficient of correlation for the particular combination plot under consideration. The intraclass coefficients of correlation have been calculated by Harris' method, and the equation is given later. Table 5, column 8, records the intraclass correlations used in ascertaining the theoretical coefficients of variation in column 6. Now, columns 5 and 6 agree—a fact brought out very clearly by figure 5. Change in variability in a closed system of this kind is therefore a function of the number of ultimate plots combined and their intraclass correlation. General disregard of the intraclass correlation unquestionably accounts for the poor agreement between actual and theoretical curves.

TABLE 5.—*Mean, standard deviation, coefficient of variation, and intraclass correlation coefficient of grain yield for rows of various lengths, when the entire area is utilized for each row length* [1]

Length of row (feet)	Rows	Mean yield per row	Standard deviation	Coefficient of variation			Value of intra-class correlation
				Actual	Theoretical when $r_p \neq 0$	Theoretical when $r=0$	
	Number	*Grams*	*Grams*				
15	1,500	587.89±[2] 2.58	100.09±[2] 1.83	17.03±[2] 0.32	17.03±[2] 0.32	17.03±[2] 0.32	
30	750	1,175.30± 6.55	179.32± 4.63	15.25± .40	15.25± .40	12.03± .32	+0.604
45	500	1,761.46±11.56	258.54± 8.18	14.66± .47	14.66± .47	9.83± .32	+.611
60	375	2,350.18±16.51	319.74±11.68	13.59± .51	13.59± .51	8.51± .31	+.516
90	250	3,526.82±28.71	453.94±20.30	12.86± .58	12.86± .58	6.95± .31	+.484
180	125	7,054.50±56.76	634.56±40.13	9.00± .57	9.00± .57	4.91± .31	+.214

[1] The rows were lengthened by adding together the yields from rows which had their ends adjacent to each other.
[2] Standard error.

CORRELATION BETWEEN ROWS AT DIFFERENT DISTANCES APART

In the previous section, the marked effect of the coefficient of intraclass correlation upon the variability between combination plots was pointed out. Student (*1*) makes the following statement: "The art of designing all experiments lies even more in arranging matters so that r_{ab} (the correlation between paired plots) is as large as possible, than in reducing σ_a^2 and σ_b^2." With this in mind, a study was made of the correlation between the ultimate plots when the distance between them was varied.

The method of calculating the correlations is as follows. Suppose the correlation coefficient between rows 8 feet apart is desired. It may be designated r_{19} because the distance between rows numbered 1 and 9 is 8 feet. The correlation table is entered with rows 1 and 9 of series 1 as the first pair, rows 9 and 17 as the second pair, rows 17 and 25 as the third pair, etc. Thus there were 15 pairs for each series, or 180 pairs for the 12. The procedure of arriving at pairs in the other series was the same as for series 1.

As noted above, the rows were sown with a grain drill that seeded eight rows at a time. At harvest time, it was noted that the distance between some of the rows was not exactly 12 inches. The result was a small amount of systematic variation within a drill width. Some variations also occurred in the distance between rows where one drill width of rows bordered another. To avoid these systematic errors, no outside row of any drill width was used as a member of a pair in the present correlation study. Actually, in working out r_{19}, the correlation table was entered with the first pair as rows 3 and 11, the second as 11 and 19, the third as 19 and 27, etc. For r_{14} the first pair was rows 3 and 6, the second 11 and 14, the third 19 and 22, etc. All the coefficients were calculated for rows 15 feet long.

After the correlation scatters had been made they were coded, and r was computed by the following formula:

$$r=\frac{\sigma_1^2+\sigma_2^2-\sigma_{1-2}^2}{2(\sigma_1\ \sigma_2)}$$

A calculating machine and a short-cut method reduced the labor to a minimum.

The values for r obtained at different distances are recorded in table 6 and are shown graphically in figure 6. Most of the coefficients calculated were on distances that lie in a north-south direction. To see whether an east-west direction would give a different value, the coefficients for 18- and 36-foot distances were calculated. These are

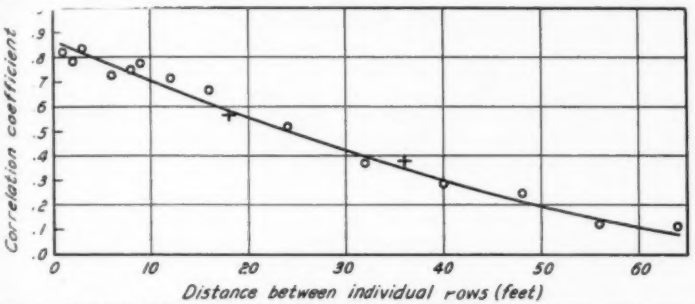

FIGURE 6.—Correlation between grain yields of 15-foot rows at different distances apart. The circles (o) indicate correlations when the distance between rows was in a north-south direction, and the crosses (+) when the distance was east-west. The line drawn to fit the points is a "freehand" curve.

shown as crosses (+) in figure 6; the circles (o) are for coefficients in a north-south direction.

TABLE 6.—*Correlation between grain yield of the ultimate rows (15 feet in length) when the distance separating them is varied*

Direction and distance between rows (feet)	Correlation coefficient	Direction and distance between rows (feet)	Correlation coefficient
North to south		*North to south*	
1	+0.821	32	+0.366
2	+.781	40	+.291
3	+.828	48	+.246
6	+.721	56	[1] +.179
8	+.750	64	[1] +.112
9	+.773	*East to west*	
12	+.710		
16	+.667	18	+.569
24	+.516	36	+.381

[1] Not significantly different from zero.

Figure 6 shows that the correlation falls off as the distance between rows increases. The line drawn to fit the points in this figure is a freehand curve, not one fitted by statistical methods. The correlation decreases at an almost uniform rate as the distance increases. The two east-west values shown as (+) fit very closely to what would be expected on a north-south basis. The values calculated at the 56- and 64-foot distances are +0.179 and +0.112, respectively, but these do not differ significantly from zero. All the other values are significant. The relationship shown here for the value of r and the distance between rows is discussed again later.

INTRACLASS CORRELATION AND VARIATION WITHIN COMBINATION PLOTS COMPOUNDED BY DIFFERENT REPLICATION SYSTEMS

The term "replication" is used here in its broadest sense. Forming a combination plot from two adjacent ultimate plots is considered replication just as much as if the plots had been distributed by some random or controlled system and at a greater distance from each other.

For this study the last 5 rows of each series were not used, so that 120 rows remained in each series. This was done because 120, an even number, is exactly divisible by more numbers than is 125. The number of rows used, therefore, was 1,440 instead of 1,500.

The relationships of replication to intraclass correlation and variation within combination plots are considered under two aspects. (1) When the replicates are distributed by contiguous association, one may conveniently speak of size and shape of combination plot in referring to number and arrangement of the contiguous ultimate plots, to whose intraclass correlation and variation they have an important relation. (2) When the replicates are distributed noncontiguously, they may be considered in two ways: (*a*) Completely at random and (*b*) at random within certain established limits.

Contiguous Distribution of Replicates

In evaluating the intraclass correlation and variation within combination plots, the intraclass correlation coefficient developed by Harris (*10*) and the analysis of variance by Fisher (*5*) were used. The equation for computing the value for the intraclass correlation coefficient r follows:

$$r = \frac{\{[S(C_p^2) - S(p^2)] \div m[n(n-1)]\} - \bar{p}^2}{\sigma_p^2}$$

where

r = mean correlation between the yields of all the ultimate plots within a combination plot.
C_p = total yield of a combination plot.
p = yield of an ultimate plot.
m = number of combination plots.
n = number of plots within a combination plot.
$\bar{p}$ = mean yield of the ultimate plots.
σ_p = standard deviation of the ultimate plots.
S = summation.

To illustrate the meaning of r obtained by this method, a combination plot formed from 3 adjacent rows is used. There are then $\frac{n(n-1)}{2}$ or 3 pairs of yields to be entered in the correlation table, namely, yield of row 1 with yield of row 2, 1 with 3, and 2 with 3. If the pairs are entered also as 2 with 1, 3 with 1, and 3 with 2, the correlation table is made symmetrical. This reciprocal method of entry gives $n(n-1)$ pairs of entries for each combination plot, and thus the entries are made for all the other combination plots. Thus, r is the mean correlation coefficient between the yields of all the ultimate plots within a combination plot. A combination plot may be defined as one made by adding together the desired number of ultimate plots. For example, a plot having a width : length ratio of 4/30=0.1333 and a size of 8 rows is formed by adding rows 1 to 4 of series 1 to rows 1 to 4 of series 2. The second plot is made from rows 5 to 8 of series 1 plus rows 5 to 8 of series 2, and so on for the other combination plots.

The intraclass correlation coefficients were calculated for a number of plot sizes and shapes and are given in tables 7 and 8. Table 7 gives the value of r for varying plot sizes when certain definite plot

shapes are considered. In table 8 the value of r is for varying plot shapes when certain definite plot sizes are considered. The data show that the correlation becomes less with an increase in size when any particular shape of plot is considered, and for a constant size becomes less as the plot becomes long and narrow.

TABLE 7.—*Relation of size of plot to intraclass correlation coefficient and to coefficient of variation of yield of grain within and between combination plots compounded by contiguous association, when different shaped plots are considered*

Shape of plot width/length	Combination plots	Size of plot		Intraclass correlation coefficient	Coefficient of variation	
		Rows	Area		Within combination plots	Between combination plots
Feet Coefficient	*Number*	*Number*	*Sq. ft.*			
15/15=1.0000	96	15	225	+0.660	10.00±[1]0.19	14.18±[1]1.04
30/30=1.0000	24	60	900	+.469	12.50± .23	12.14± 1.78
8/15= .5333	180	8	120	+.681	9.70± .18	14.56± .78
24/45= .5333	20	72	1,080	+.477	12.40± .25	12.25± 1.97
4/15= .2667	360	4	60	+.710	9.30± .17	15.15± .58
8/30= .2667	90	16	240	+.545	11.57± .22	12.99± .98
12/45= .2667	40	36	540	+.532	11.74± .22	12.66± 1.44
24/90= .2667	10	144	2,160	+.350	13.83± .26	10.77± 2.44
2/15= .1333	720	2	30	+.679	9.72± .18	15.72± .42
4/30= .1333	180	8	120	+.564	11.32± .21	13.50± .72
8/60= .1333	45	32	480	+.456	12.66± .24	11.79± 1.26
12/90= .1333	20	72	1,080	+.398	13.32± .25	11.21± 1.79
1/15= .0667	[2]1,440	1	15			17.16± .33
2/30= .0667	360	4	60	+.558	11.46± .22	13.99± .53
4/60= .0667	90	16	240	+.471	12.47± .24	12.18± .92
6/90= .0667	40	36	540	+.413	13.15± .25	11.24± 1.27
12/180= .0667	10	144	2,160	+.130	16.00± .31	6.68± 1.50
1/30= .0333	720	2	30	+.604	10.71± .20	15.26± .41
2/60= .0333	180	8	120	+.516	11.93± .23	13.03± .70
3/90= .0333	80	18	270	+.439	12.86± .24	11.77± .94
6/180= .0333	20	72	1,080	+.143	15.88± .30	6.92± 1.10
1/45= .0222	480	3	45	+.611	10.62± .20	14.68± .48
1/60= .0167	360	4	60	+.516	11.85± .22	13.60± .52
1/90= .0111	240	6	90	+.484	12.23± .23	12.87± .60
1/180= .0056	120	12	180	+.209	15.26± .29	9.00± .59

[1] Standard error. [2] Number of ultimate plots.

TABLE 8.—*Relation of shape of plot to intraclass correlation coefficient and to coefficient of variation of yield of grain within and between combination plots compounded by contiguous association, when different sized plots are considered*

Size of plot		Shape of plot width/length	Combination plots	Intraclass correlation coefficient	Coefficient of variation	
Rows (number)	Area				Within combination plots	Between combination plots
	Square feet	*Feet Coefficient*	*Number*			
2	30	2/15=0.1333	720	+0.679	9.72±[1]0.18	15.72±[1]0.42
2	30	1/30= .0333	720	+.604	10.71± .20	15.26± .41
4	60	4/15= .2667	360	+.710	9.30± .17	15.15± .58
4	60	2/30= .0667	360	+.554	11.46± .22	13.99± .53
4	60	1/60= .0167	360	+.516	11.85± .22	13.60± .52
8	120	8/15= .5333	180	+.681	9.70± .18	14.56± .78
8	120	4/30= .1333	180	+.564	11.32± .21	13.50± .72
8	120	2/60= .0333	180	+.516	11.93± .23	13.03± .70
16	240	8/30= .2667	90	+.545	11.57± .22	12.99± .98
16	240	4/60= .0667	90	+.471	12.47± .24	12.18± .92
36	540	12/45= .2667	40	+.532	11.74± .22	12.66± 1.44
36	540	6/90= .0667	40	+.413	13.15± .25	11.24± 1.27
72	1,080	24/45= .5333	20	+.477	12.40± .23	12.25± 1.97
72	1,080	12/90= .1333	20	+.398	13.32± .25	11.21± 1.79
72	1,080	6/180= .0333	20	+.143	15.88± .30	6.92± 1.10
144	2,160	24/90= .2667	10	+.350	13.83± .26	10.77± 2.44
144	2,160	12/180= .0667	10	+.130	16.00± .31	6.68± 1.50
1	15	1/15= .0667	[2]1,440			17.16± .33

[1] Standard error. [2] Number of ultimate plots.

The trends in the value of the correlation are those expected on the basis that ultimate plots farther apart are less highly correlated. When two combination plots have the same shape, the ultimate plots of the larger are farther removed from each other than are those of the smaller. And since the value of r is based on all the possible $n\,(n-1)$ pairs of the ultimate plots compounding a combination plot, a smaller value from the larger combination plot is expected, which is in fact the case. The same reasoning holds true when the combination plot remains constant in size but changes in shape. In the long narrow plot the distance between ultimate plots that go to make up all the pairs is again greater than for a relatively square plot, and hence a lower value for r is to be expected.

All the plot shapes given in tables 7 and 8, when not square, had their greatest length in an east-west direction.

The variability of the ultimate plots within a combination plot is determined by two different methods that give identical results. In one, Harris' intraclass coefficient is used in the equation

$$\sigma_w^2=\sigma_p^2(1-r)$$

where σ_w is the standard deviation of the ultimate plots within a combination plot; σ_p the standard deviation of all the ultimate plots, secured by dividing the squared deviations by n; and r the intraclass correlation coefficient. The other method is Fisher's (*5*) analysis of variance. The principle will not be described here, being found in texts by Fisher (*5*) and Tippett (*23*). To illustrate the method, however, the variability within a combination plot of 16 ultimate plots and with a width : length ratio of $8/30=0.2667$ was computed. These data are shown in table 9. The second line of the table refers to that part of the variability which is within combination plots. The standard deviation found was 68.03, or 11.57 percent of the mean value of the ultimate plots. The equation

$$\sigma_w^2=\sigma_p^2(1-r)$$

gives an identical value.

The coefficients of variation within combination plots are given in column 6 of tables 7 and 8. Table 7 gives the variability within plots for varying sizes when certain definite shapes are considered. Variability within combination plots decreases with a decrease in size when the shape remains the same. This result might be expected because contiguous plots tend to yield alike. As the intraclass correlation coefficient increases in value, the variability decreases within the combination plot. This follows from the relationships in the equation

$$\sigma_w^2=\sigma_p^2(1-r).$$

Table 8 shows the data arranged with size as the constant factor but with shape allowed to vary. Here the intraclass correlation increases and the variability within combination plots decreases as the shape of the latter approaches a square.

The data show that the size and shape of combination plots have an important bearing on the intraclass correlation and variation

within them. Hence, when studying the size of combination plots in relation to the intraclass correlation and the variation within them, one should keep the shape constant. Conversely, when shape is being studied, the size should be held constant.

TABLE 9.—*Analysis of variance of grain yield between and within combination plots compounded from 16 ultimate plots and having a width : length ratio of 8/30=0.2667*

Variation	Degrees of freedom	Sum of squares	Mean square	Standard deviation	Coefficient of variation
Between combination plots	89	8,393,855	94,313.0	307.10	12.99
Within combination plots	1,350	6,248,202	4,628.3	68.03	11.57
Total	1,439	14,642,057	10,175.2	100.87	17.16

NONCONTIGUOUS DISTRIBUTION OF REPLICATES

The only difference in studying intraclass correlation and variation within combination plots under this system and under the one just considered is in the distribution of the replicates. In the former study, where plot size and shape were emphasized, the replicates were distributed by contiguous association. In the present case, the replicates are distributed by noncontiguous association. The study has been carried out with two types of distribution: The replicates are distributed (1) completely at random and (2) at random within certain arbitrary limits. For 24 replicates, as an example, under the latter system of distribution, each of the 12 series was divided so that rows 1 to 60 constituted one group and rows 61 to 120 another. This gave 24 groups in all. One replicate then fell at random within each of these groups. Under the first system of distribution the replicates fell completely at random within the 1,440 rows used.

The data secured from this study appear in table 10. Under both systems of distributing replicates, the variation within combination plots of all sizes is always higher than 17.16, the coefficient of variation for the 1,440 ultimate plots in the study. This result was surprising, as the coefficients had been expected to vary around 17.16 as a mean. The explanation is that in every case a small negative intraclass correlation exists, which, when considered, enables one to calculate a theoretical figure agreeing with the one actually found. When the replicates are distributed completely at random, none of the intraclass coefficients, though all are negative in sign, is significantly different from zero. When, however, the replicates are distributed at random within arbitrary limits, all the intraclass correlations are again negative in sign and significantly so. The significance of the intraclass correlations was tested by the z test of Fisher (*5*).

For replicates distributed at random within arbitrary limits, the trend is definitely toward reducing both the coefficient of variation and the intraclass correlation coefficient as the number of replicates is increased. Perhaps no such trend exists when the replicates are distributed completely at random.

TABLE 10.—*Actual and theoretical coefficients of variation of grain yield within and between combination plots, and intraclass correlation coefficients, when the replicates are distributed (1) completely at random and (2) within certain arbitrary limits*

REPLICATES DISTRIBUTED COMPLETELY AT RANDOM

Replicates (number) [1]	Coefficient of variation within combination plots			Coefficient of variation between combination plots			Intraclass correlation (r)	Is r significant? (z test)
	Actual	Theoretical when $r \pm 0$	Theoretical when $r = 0$	Actual	Theoretical when $r \pm 0$	Theoretical when $r = 0$		
1				17.16±[2]0.33				
3	17.39±[2]0.33	17.39±[2]0.33	17.16±[2]0.33	9.63± .31	9.63±[2]0.31	9.90±[2]0.32	−0.0275	No.
6	17.24± .33	17.24± .33	17.16± .33	6.82± .31	6.82± .31	7.00± .32	−.0104	No.
12	17.24± .33	17.24± .33	17.16± .33	4.67± .30	4.67± .30	4.95± .32	−.0099	No.
24	17.19± .33	17.19± .33	17.16± .33	3.35± .31	3.35± .32	3.50± .32	−.0036	No.
48	17.21± .33	17.21± .33	17.16± .33	2.06± .27	2.06± .27	2.48± .32	−.0066	No.
60	17.22± .33	17.22± .33	17.16± .33	1.70± .25	1.70± .25	2.21± .32	−.0070	No.
180	17.18± .33	17.18± .33	17.16± .33	.89± .22	.89± .22	1.28± .32	−.0029	[3]

REPLICATES DISTRIBUTED WITHIN CERTAIN ARBITRARY LIMITS

1				17.16±[2]0.33				
2	18.71±[2]0.36	18.71±[2]0.36	17.16±[2]0.33	10.92± .29	10.92±[2]0.29	12.13±[2]0.32	−0.1898	Yes.
3	18.03± .35	18.03± .35	17.16± .33	8.80± .29	8.80± .29	9.90± .32	−.1052	Yes.
4	17.76± .34	17.76± .34	17.16± .33	7.60± .28	7.60± .28	8.58± .32	−.0716	Yes.
6	17.66± .34	17.66± .33	17.16± .33	5.88± .27	5.88± .27	7.00± .32	−.0592	Yes.
12	17.47± .34	17.47± .34	17.16± .33	3.81± .25	3.81± .25	4.95± .32	−.0370	Yes.
60	17.26± .33	17.26± .33	17.16± .33	1.15± .17	1.15± .17	2.21± .32	−.0124	Yes.
180	17.18± .33	17.18± .33	17.16± .33	.87± .22	.87± .22	1.28± .32	−.0030	[3]

[1] Number of replicates refers to the actual number of ultimate plots compounding a combination plot.
[2] Standard error.
[3] The number of combination plots is too small for an accurate test.

The existence of negative intraclass correlation coefficients has more than a passing interest. The negative correlation results from a reduction of the product moment in the equation for the calculation of r. That a negative r exists for combination plots compounded from two ultimate plots selected at random within arbitrary limits is shown by the contour map (fig. 2). Here the field was divided so that series 1 to 6 constituted one group and series 7 to 12 the other. The map shows that as combination plots are made by selecting at random one plot from each group, a high-yielding plot of the first group may often be combined with a relatively low-yielding plot in the second. When such pairs are entered in a correlation table in excess of the normal amount as expected from a normal distribution solely on the basis of chance, then a negative value is obtained. Perhaps when the means of the two groups differ significantly, a negative intraclass correlation coefficient follows. In the particular case considered, the means did differ significantly, and the value of -0.1898 for r (table 10) is significantly different from zero.

VARIATION BETWEEN COMBINATION PLOTS COMPOUNDED BY DIFFERENT REPLICATION SYSTEMS

Sometimes larger units than the ultimate one used in these experiments are desired. To investigate this phase of the problem the coefficients of variation for the larger plots were calculated. As in the preceding section, it is convenient to treat the variation between combination plots when they are formed (1) from contiguous ultimate plots and (2) from noncontiguous ultimate plots. In the

former instance size and shape may conveniently be discussed in relation to variation, and in the latter the number and the systems of distribution of ultimate plots, as replicates in relation to variation.

Contiguous Distribution of Replicates

It is to the variability between combination plots that most investigators have devoted their attention. In order to study this phase of the problem, the coefficients of variation were calculated for different sizes and shapes of combination plots. The data for the relation of size to variability, with shape constant, are given in table 7, and for the relation of shape to variability, with size constant, in table 8. The data show that the variability diminished as the size of plot was increased for any particular shape, and also as the shape of plot became long and narrow for any particular size. The results

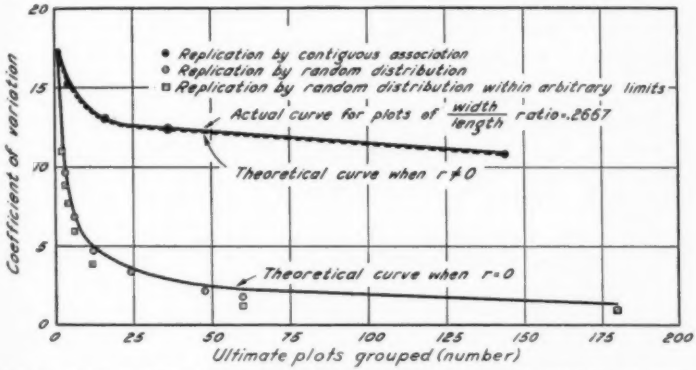

Figure 7.—Coefficient of variation between combination plots in relation to replication by contiguous and noncontiguous association for various numbers of ultimate plots grouped. It is possible to calculate two additional theoretical curves, one of which will pass through the circles and the other through the squares, representing the reduction in variation under two different systems of noncontiguous replication for various numbers of ultimate plots grouped.

emphasize the importance of keeping shape constant when studying size and size constant when studying shape.

The upper line (solid) of figure 7 shows the relationship between size of combination plot and variation for a particular plot shape of the width: length ratio=0.2667. For simplicity's sake the curves for the other plot shapes are not shown. They are, however, similar in type. The lower line (solid) (fig. 7) is the theoretical curve expected on the basis that the reduction in variation is proportional to the square root of n, the number of ultimate plots grouped. A wide discrepancy appears between the two curves. However, by allowing for the correlation that exists among the ultimate plots compounding the combination plots, a second theoretical curve can be constructed which agrees perfectly with the actual results obtained. This curve is the dotted line just below the solid line in figure 7. Actually the two curves are identical, but on the planar graph they had to be drawn parallel to each other. The reduction in variation is therefore a function of n, the number of ultimate plots grouped, and of r, their intraclass correlation coefficient. The equation for computing the variation as a function of n and r has already been cited.

Noncontiguous Distribution of Replicates

The usual practice in agronomic field experiments is to distribute several replicates of the treatments or varieties over the area on which the tests are made. In studying this type of procedure, combination plots were compounded with different numbers of replicates or ultimate plots distributed in different ways. Of the many possible systems of distributing the replicates only two are investigated in this study: (1) That in which distribution is completely at random and (2) that in which distribution is at random within certain arbitrary limits. Replicates may fall next to each other by chance, but such cases are exceedingly few and will not influence the results to any marked degree.

The results of this study appear in table 10. The data show that the reduction in variation, as the number of replicates is increased, is much greater under this system than where the replicates are distributed by contiguous association. This fact is shown graphically in figure 7. The reduction in variation is nearly proportional to the square root of the number of replicates used. In every case investigated, the actual reduction of variation was slightly greater than that expected on the basis of a zero correlation coefficient between the replicates of the combination plots. These discrepancies are caused by the existence of small negative intraclass correlation coefficients, the values for which are given in table 10. When these coefficients are considered, the theoretical figure for the variation agrees exactly with the one actually obtained. Table 10 shows this in columns 5 and 6. Two theoretical curves can therefore be calculated, one passing through the circles and the other through the squares of figure 7.

When the same number of replicates was investigated under both systems of distribution mentioned above, the reduction in variation was always slightly greater where the replicates were distributed at random within certain arbitrary limits. The difference is explained by the value of the intraclass correlation coefficient, the only factor left to vary when two systems involving the same number of replicates are compared. If the equation for computing the intraclass correlation coefficient is examined, the only variable that changes, r, evidently is the product moment part, as all the other variables remain constant. Hence, if one can explain why the product moment changes for different systems of distribution, the difference in variation follows logically. For the sake of brevity, it will suffice to show how the product moments differ for the 2 systems when only 2 replicates are considered. Suppose the 2 replicates compounding a combination plot yielded alike, say 500 g each. Then the product (500×500) will be a maximum. Any other division of the yields of the replicates, say 490 and 510, will give a product of lower numerical value. This unequal division of the replicates compounding a combination plot (an inequality too great to be caused by chance alone in drawing at random from a normally distributed population) probably explains the difference in the product moments. To illustrate more specifically, let the first replicate be selected from series 1 to 6, the second from series 7 to 12, both selections being at random within these areas. Now the mean yield of the ultimate plots of series 1 to 6 is 625.22 ± 3.77 g; of series 7 to 12, 550.55 ± 2.96 g. The difference (74.67 ± 4.79 g) is highly significant. The product moment formed by selecting the

first replicate from a group ranging around 625.22 as a mean and the second from one ranging around 550.55 is less than the product moment from two replicates selected at random around the grand mean of 587.89 ± 2.58 g. Obviously, the average difference between the means of the first and second replicates, each selected completely at random, would not exceed some 15 times its standard error except in a very large number of trials.

As shown earlier in this paper, adjacent rows are positively correlated, and significant positive correlations are found between rows up to a distance of 48 feet apart. Now the establishment of arbitrary limits on a field of this kind tends to make the variation of the means (derived from the ultimate plots within the limits) greater than if the same number of ultimate plots were selected at random. Since the means vary more, the product moment of the intraclass correlation coefficient of single replicates selected at random from within these delimited groups will be less, for the reasons just stated. Hence the variation between combination plots compounded by a systematic distribution of the replicates will usually be less than where the replicates are distributed completely at random.

THE PRINCIPLE OF MAXIMUM CONTIGUITY AND ITS PRACTICAL APPLICATION

According to Student (*1, pp. 274–275*), the principle of maximum contiguity in field-plot technic was deduced a priori by E. S. Beaven. Although the principle is not formally defined, its essence is contained in the following statement by Student:

> * * * naturally the nearer two plots are together the more likely is the soil and its condition to be similar on each of them, and the obvious conclusion may be drawn that the smaller the plots the more exactly can the yield of adjacent plots be compared.

Applied to the data under consideration, the principle may be stated as follows: When the yields of nearby plots are correlated, the mean distance between the contiguous ultimate plots compounding combination plots has an influence on the variability within and between the latter. Hence the nearer the ultimate plots are together in a combination plot of a given size, the less will be the variability between these plots.

The question as to the best size and shape of plot on which to place a single replication of a number of varieties may be approached on the principle of maximum contiguity, assuming that the variance between replications can be eliminated by methods given by Student (*1*), Fisher (*5*), or Engledow and Yule (*4*). Suppose three varieties of wheat are to be tested for their yielding ability. In the United States, the ultimate unit of small-scale testing is usually a three-row block, 15 to 18 feet in length, of which only the center row is harvested. The rows are usually 12 inches apart, and sometimes their ends are discarded before cutting to eliminate any border effect. A total of nine rows then will be needed to test the three varieties, which are distributed at random in each replication. In table 8 the nearest size of plot investigated contains eight rows. Using this size in ascertaining the best arrangement in which to grow the rows, one finds that the coefficient of variation of the rows within the plot for this size decreases in proceeding from a long narrow plot (width: length ratio 0.0333) to one more nearly square (width: length ratio 0.5333). The actual coefficients are 11.93, 11.32, and 9.70. Obvi-

ously, the plot with the smallest coefficient (9.70) would be best for testing the three varieties.

As another example, suppose 24 varieties are to be tested. This will require a plot of 72 rows. Three shapes have been investigated for this size, with results shown in table 8. Here again, the more nearly square the plot the smaller will be the coefficient of variation. Under the restriction that all the land had to be used whenever a particular grouping was studied, a plot shape more nearly square could not be made for a size of 72 rows. If the general trend of variability is at all indicative, one would expect a lower figure even than 12.40 (this is for a width: length ratio of 0.5333) for a square plot.

The following general procedure seems to be a practical application of the principle of maximum contiguity when the yields of numerous varieties are to be tested. As previously shown in this study, the variation of ultimate plots within a combination plot compounded from contiguous replicates of a given number decreases as the combination plot changes in shape from long and narrow to square. Within square combination plots, the variation of the ultimate plots decreases as their number decreases. One must therefore select the number of varieties that are to be grouped into one replication of the test, and then arrange them to form a combination plot as nearly square as is practicable. In the present case, five varieties would seem a desirable number to select. One replication of these would occupy 15 ultimate plots, and they could, by the principle of maximum contiguity, be arranged into a square combination plot. In order not to introduce a bias in estimating the experimental error, the varieties in any one replication will be assumed to be arranged at random. All the varieties, in groups of five, can then be investigated as to yielding ability. It seems also advisable to include in each group of five a standard or check variety which is the same for each group. The experimental error is perhaps best estimated by an analysis of variance, deducting the variance due to replications and varieties from the total variance in arriving at the experimental error. If a very large number of varieties are to be tested, the experimental error need not be calculated for each group of 5; but the average error from several groups of 5 may be applied throughout all the groups of 5 varieties. The only valid use of the error is, of course, in comparing the yields of the five varieties in a group. Of particular interest is the comparison of the standard or check variety with each of the four other varieties in the group.

In another plan that seems to have some points of merit, the varieties are planted in sequence 1, 2, 3, . . . n, and repeated in like manner for all subsequent replications. Every fifth, tenth, fifteenth, twentieth variety, etc., is a standard variety or check. The experimental error is estimated in the same manner as in the preceding case. The present arrangement permits the error to be applied as a "moving error" for any contiguous group of five varieties. For instance, the average yields of varieties 6, 7, 8, 9, 10 may be compared with each other. The same is true of varieties 7, 8, 9, 10, 11, 12; 9, 10, 11, 12, 13, etc. The "moving error", which has the same percentage value throughout the experiment, permits each of the four varieties between the checks to be compared with both those checks. For example, variety 8 can be compared with the check varieties at 5 and 10. Often two check varieties are used alter-

nately, as when the hybrids of a cross are compared with the parents. The type of planting suggested here is well suited for that purpose. The efficacy of this method to reduce the experimental error depends on the value of the intraclass correlation coefficient. A valid objection to this planting scheme is that it is systematic and may therefore introduce some bias into the experimental error. A slight bias in the error might, however, be easily outweighed by other practical considerations, such as ease in planting, harvesting, etc. Further studies on this point are needed.

DISCUSSION

When the general variability in grain yield within a field is investigated by adding more plots of the same size and shape to enlarge the field, Stringfield (*20*) and Stadler (*18*) showed that the total variation has a tendency to increase. In this paper such a procedure is called the extended system of sampling. Stringfield studied the variation of increasing field sizes when the plot sizes varied from those customary in nursery work to 0.1 acre. In all cases, he found the total variation increased as more and more land was brought into the experimental area. Both wheat and oats were used in his studies. Stadler, working with nursery plots of the same grains, had similar results. The data of the present study agree with those of Stringfield and Stadler. Since the variation and mean yield of the plots to be added to the experimental area are unknown, the variation of the enlarged area cannot be predicted. Apparently the only conclusion warranted by the data at hand and by those of others (*18*, *20*) is that the total variation will perhaps be greater for the larger area than for the smaller one. Change in variation of increased areas depends on the relation of the mean and variation of the additional plots to those already in the experiment.

Under another type of extended system, where more and more land is brought into the experimental area by increasing the size (in this study, lengthening the individual rows) of the original plots, the total variation tends to decrease. Stringfield (*20*) studied a comparable case with similar results. Here, again, the actual variation cannot be predicted beforehand, because the character of the land added is not known. In part, at least, the reduction occurs because positive and negative deviations cancel each other as adjacent plots are added together and because the variability of a series of averages (adjacent plots added together) tends to be less than that of the single plots.

When the variability of different row lengths is investigated and all the land is used for each study (the closed system), the variability decreases as the rows become longer. From a practical viewpoint of field-plot technic the question might be asked, what is the best length of row to use? The length of row in nursery practice has usually been determined by convenience: 16 feet for wheat, 15 for oats, and 20 for barley. The yields from these row lengths, taken in grams, are easily converted into bushels per acre. It seems rather anomalous that the row lengths in common practice should have been selected on such a criterion. As shown by figure 5, the curve for the coefficient of variation is almost a straight line, although the reduction in going from a 15-foot to a 30-foot row is a little greater than for any other part of the curve. The present study does not seem to justify a departure from the row lengths now used.

The correlation between adjacent rows and those at greater distances has been investigated by Hayes (*12*) and Griffee (*8*). Hayes, studying wheat and oats, computed the correlation for rows separated by 3, 6, 9, 12, 15, and 30 feet, respectively. He found that the correlation decreased as the distance between rows was increased. Griffee, working with wheat, calculated the values of r for rows up to 10 feet apart. Though this is only a short distance, still the values he found seemed to be smaller as the distance was increased. On the whole, the values obtained in the present study agree with those of Hayes and Griffee. They are higher for comparable distances between rows, but this difference may not be significant, as there is not a priori reason why they should be equal. A possible influencing factor may be that Hayes and Griffee correlated row lengths slightly longer than those in this study; they used rod rows, whereas in this study the row lengths were 15 feet. According to the data at hand, the value of r diminished as the distance between plots increased; and the resulting curve, shown in figure 6, is very nearly a straight line. Significant correlations were not demonstrated beyond 48 feet.

After Harris (*10*, *11*) developed a short-cut method for calculating the intraclass correlation between the ultimate units compounding a combination plot, this feature of field-plot technic could be studied. The method is applicable to combination plots where the replicates are distributed either contiguously or noncontiguously. The former distribution has been used in the most extensive studies made. Harris has devoted a good deal of attention to this matter. He computed the coefficients from a relatively large number of uniformity trials distributed throughout the world and planted to a considerable variety of crops. The coefficients ranged from $+0.020$ to $+0.830$ for adjacent plots. Harris was able to show that the correlation decreased as the number of ultimate plots in a combination plot increased. Garber, McIlvaine, and Hoover (*7*), working with wheat, also found the correlation to decrease as the number of ultimate plots in a combination plot increased. The data from the present study agree with those just mentioned (*7*, *10*, *11*). For the particular crop studied (wheat) they have been extended to cover a greater range of combination plot size and shape. Heretofore, plot shape has been largely disregarded when the intraclass correlation for varying sizes was being studied. These experiments emphasize the fact that plot shape is an important factor in determining the numerical value of the coefficient. For any given size of combination plot the intraclass correlation coefficient became greater as the shape approached a square. The data showing these relationships are given in tables 8 and 9.

The variation of the ultimate plots within a combination plot has a simple relation to the intraclass correlation coefficient, as is shown by Fisher (*5*). The relation is an inverse one; that is, as the correlation of the ultimate plots of a combination plot increases, their variation decreases. The variation, therefore, decreases when the shape is constant but the size decreases, and similarly when the size is constant but the shape approaches a square (tables 7 and 8).

The intraclass correlation coefficient and the variation within combination plots were also calculated when the replicates were distributed noncontiguously. Garber, McIlvaine, and Hoover (*7*), investigating some wheat-yield data in this way, concluded that the intraclass correlation for replicates thus distributed was sensibly zero.

The distribution in their case was systematic. In the present study, two types of distribution were used: The replicates were distributed (1) completely at random and (2) at random within certain arbitrary limits. The resultant data are given in table 10. The intraclass correlation coefficients for both types of distribution proved to be much smaller than when the replicates were contiguous. When distribution is completely at random the values for r are not significantly different from zero, but when it is systematic they are significantly different. The variation of the ultimate plots within combination plots is in these instances also inversely related to the intraclass correlation, as indicated in the preceding paragraph. Care must be taken, however, in interpreting the present cases, as the sign of r is negative.

The variation between combination plots has received most attention from investigators. It is discussed separately for combination plots formed by contiguous and noncontiguous distribution of the replicates. Mercer and Hall (*15*), working with wheat combination plots distributed contiguously, found variability decreasing as plot size increased. Other investigators who obtained similar results with wheat are Montgomery (*16*, *17*), Forster and Vasey (*6*), Day (*3*), Summerby (*21*), Stringfield (*20*), and others. In the present study similar results were obtained; as the size of plot was increased for any particular shape, the variability decreased. The data are given in table 7. A general criticism that might be made of some earlier work is that the plot shape was not kept the same when size was investigated in relation to variability.

Lyon (*14*) was perhaps the first to point out that plot shape is an important factor in plot variability. Analyzing the data from the Mercer and Hall (*15*) wheat trial, he showed for the two plot shapes studied that long, narrow plots were more uniform than square ones. Day (*3*), studying the data from a wheat uniformity trial, reached the same general conclusions. He found the long, narrow plot of particular advantage if its greatest dimension extends in the direction of greatest soil variation. An objection to Day's analysis is that he kept his plot size only within certain limits when studying the effect of shape. Stringfield (*20*) concluded that among small-grain nursery plots increasing the length was distinctly more advantageous than increasing the width. The most extensive examination of plot shape as related to variation was made by Christidis (*2*), who critically analyzed the results of eight uniformity trials and found that in no case were square plots less variable than long, narrow ones. In certain cases, the latter shape could not be shown statistically to have any advantage, but he concluded that those cases were open to criticism because certain mechanical errors were not kept properly under control. Other investigators of wheat who have found a reduction in variability for the long, narrow plot are Summerby (*21*), Forster and Vasey (*6*), and Kiesselbach (*13*). The results of the present study (table 8) agree with those just reviewed; they show that plot variability is influenced by both size and shape, and that one must keep size constant when studying shape, and vice versa.

The variation between combination plots compounded by noncontiguous distribution of the replicates has been studied by Mercer and Hall (*15*) and many other investigators. The efficacy of this method in diminishing the variation has not been seriously challenged by any one, so far as the writer is aware. The data of this study (table 10)

confirm the results of other workers. The reduction in variation is nearly proportional to the square root of the number of replicates used.

Tedin (*22*) has investigated eight uniformity trials from different parts of the world by different systems of distributing the replicates. He found that systematic arrangements introduce a bias in the estimated error of the experiment, and in general give estimates either over or under those derived from random arrangements. The data secured in this study also agree with his findings. Figure 7 shows that the variation for noncontiguous replicates distributed at random within certain arbitrary limits is always less than where distribution is completely at random. Practical considerations may, however, be more important than the slight bias introduced. More studies are needed on this point.

Many contributors to the subject of field-plot technic have had difficulty in harmonizing their actual curves with those based on the equation where the reduction in variation between combination plots is proportional to the square root of n, the number of ultimate plots grouped. Student (*1*), Engledow and Yule (*4*), and Stadler (*19*) have pointed out that the reduction in variation is proportional to the square root of n only if the intraclass correlation is zero. Where the combination plots are compounded from contiguously distributed replicates, the intraclass correlation usually has a significant positive value and hence the variation is reduced less than expected. On the other hand, when the replicates are distributed in a systematic noncontiguous manner, the correlation is more often negative in value and the variation is reduced more than expected. The present study shows that the actual and theoretical curves agree absolutely if the latter are calculated as a function of n and r. The equation expressing the relationship is given in the text. The relation of actual and theoretical curves is shown graphically in figures 5 and 7, and the data are given in tables 5 and 10.

Like the variation between combination plots, the theoretical variation within combination plots can be computed. A different equation, however, must be employed.

SUMMARY AND CONCLUSIONS

The total variation tends to increase as more land is added to the experimental area, provided the size and shape of the ultimate unit remain the same.

The total variation tends to decrease as more land is added to the experimental area and the size of the ultimate plot increases, provided the number of plots remains the same. In this connection the size of the plot was increased by lengthening the row.

When the entire experimental area is used in each study of the relation of variation to increase in row length, the variation tends to decrease and is a function of n, the number of ultimate plots combined, and r, their intraclass correlation coefficient. The change of variation under these conditions is nearly linear.

As the distance between the rows (the ultimate plots) increases, their correlation decreases. Statistically significant correlations could not be shown beyond 48 feet.

The intraclass correlation coefficient, calculated according to a formula of Harris, increases as the size of the combination plot (compounded by contiguous association) decreases, provided the shape remains constant. When the size of plot remains the same, the coefficient increases as the shape of the plot approaches a square.

The variation of the ultimate plots (rows) within a combination plot compounded by contiguous association may be secured from the intraclass correlation coefficient with the aid of an equation or from an analysis of variance. The two methods of approach lead to the same result.

The variation within combination plots compounded by contiguous association decreases when the shape is constant but the size decreases and also when the size is constant but the shape approaches a square.

The variation within combination plots compounded by noncontiguous association approaches the total variation. The nearness of approach depends on the value of the intraclass correlation. In this study the variation within the combination plots was slightly greater than the total variation because of the small negative values for r.

The variation between combination plots compounded by contiguous association increases when their shape is constant but their size decreases and also when their size is constant but their shape approaches a square.

The variation between combination plots compounded by noncontiguous association decreases as the number of ultimate plots grouped becomes larger. The reduction in variation is nearly proportional to the square root of n, the number of plots grouped.

The decrease in variation between combination plots is greater when the replicates are distributed over the experimental area than when they are distributed by contiguous association.

The discrepancy frequently found by agronomists between actual and theoretical curves for the decrease in variation between combination plots, as more ultimate plots are used, is shown to result from the correlation between the ultimate plots compounding the combination plots. The variation is a function of n, the number of ultimate plots grouped, and r, their intraclass correlation. When n and r are both considered and the correct theoretical curve is calculated with these as the independent variables, the actual and theoretical curves agree exactly.

When the replicates were distributed in a noncontiguous manner, complete random distributions gave less bias to the estimate of the experimental error than did systematic distributions.

The data indicate that a smaller experimental error is obtained by grouping the varieties of each replication so as to make the distance separating them a minimum. The application of this method, called the principle of maximum contiguity, permits a greater part of the total variance to be apportioned to that designated as "between combination plots", which can be eliminated statistically, and thus leaves a smaller portion for calculating the experimental error.

The data suggest the use of two systems of plot arrangement for nursery practices when the number of varieties to be tested is large. These systems involve the principle of maximum contiguity. The varieties to be tested are divided into groups of 5. One of the 5 in each

group is a standard variety, or check, and the groups are replicated the desired number of times. In one system, the varieties are distributed at random in each group; in the other, they are arranged in the same sequence in the replications. An experimental error is calculated by analysis of variance for each group of 5 varieties when the number of varieties tested is not too large. When many varieties are tested, the average error from several groups of 5 may be applied throughout all the groups of 5. When the varieties are planted in sequence, the experimental error may be used as a "moving error" and applied to any contiguous group of 5 varieties. The moving error permits any variety to be compared with the check, or standard variety, on either side.

LITERATURE CITED

(1) ANONYMOUS.
1923. ON TESTING VARIETIES OF CEREALS. By Student. Biometrika 15: [271]–293, illus.

(2) CHRISTIDIS, B. G.
1931. THE IMPORTANCE OF THE SHAPE OF PLOTS IN FIELD EXPERIMENTATION. Jour. Agr. Sci. [England] 21: [14]–37, illus.

(3) DAY, J. W.
1920. THE RELATION OF SIZE, SHAPE, AND NUMBER OF REPLICATIONS OF PLATS TO PROBABLE ERROR IN FIELD EXPERIMENTATION. Jour. Amer. Soc. Agron. 12: 100–105.

(4) ENGLEDOW, F. L., and YULE, G. U.
1926. THE PRINCIPLES AND PRACTICE OF YIELD TRIALS. 78 pp., illus. London. (Empire Cotton Growing Rev., v. 3, nos. 2–3.)

(5) FISHER, R. A.
1932. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 4, rev. and enl., 307 pp., illus. Edinburgh and London.

(6) FORSTER, H. C., and VASEY, A. J.
1929. EXPERIMENTAL ERROR OF FIELD TRIALS IN AUSTRALIA. Jour. Dept. Agr. [Victoria] 27: [385]–395, illus.

(7) GARBER, R. J., McILVAINE, T. C., and HOOVER, M. M.
1926. A STUDY OF SOIL HETEROGENEITY IN EXPERIMENT PLOTS. Jour. Agr. Research 33: 255–268.

(8) GRIFFEE, F.
1928. CORRECTING YIELDS IN ROD-ROW TRIALS WITH THE AID OF THE REGRESSION EQUATION. Jour. Amer. Soc. Agron. 20: 569–581.

(9) HALL, A. D., and RUSSELL, E. J.
1911. FIELD TRIALS AND THEIR INTERPRETATION. Jour. Bd. Agr. [London] Sup. 7: [5]–14, illus.

(10) HARRIS, J. A.
1915. ON A CRITERION OF SUBSTRATUM HOMOGENEITY (OR HETEROGENEITY) IN FIELD EXPERIMENTS. Amer. Nat. 49: 430–454, illus.

(11) ———
1920. PRACTICAL UNIVERSALITY OF FIELD HETEROGENEITY AS A FACTOR INFLUENCING PLOT YIELDS. Jour. Agr. Research 19: 279–314.

(12) HAYES, H. K.
1925. CONTROL OF SOIL HETEROGENEITY AND USE OF THE PROBABLE ERROR CONCEPT IN PLANT BREEDING STUDIES. Minn. Agr. Expt. Sta. Tech. Bull. 30, 21 pp.

(13) KIESSELBACH, T. A.
1918. STUDIES CONCERNING THE ELIMINATION OF EXPERIMENTAL ERROR IN COMPARATIVE CROP TESTS. Nebr. Agr. Expt. Sta. Research Bull. 13, 95 pp., illus.

(14) LYON, T. L.
1912. SOME EXPERIMENTS TO ESTIMATE ERRORS IN FIELD PLAT TESTS. Amer. Soc. Agron. Proc. (1911) 3: 89–114, illus.

(15) MERCER, W. B., and HALL, A. D.
1911. THE EXPERIMENTAL ERROR OF FIELD TRIALS. Jour. Agr. Sci. [England] 4: [107]–132, illus.

(16) MONTGOMERY, E. G.
1912. VARIATION IN YIELD AND METHODS OF ARRANGING PLATS TO SECURE COMPARATIVE RESULTS. Nebr. Agr. Expt. Sta. Ann. Rept. (1911–12) 25: 164–180, illus.

(17) ———
1913. EXPERIMENTS IN WHEAT BREEDING: EXPERIMENTAL ERROR IN THE NURSERY AND VARIATION IN NITROGEN AND YIELD. U. S. Dept. Agr., Bur. Plant Indus. Bull. 269, 61 pp., illus.

(18) STADLER, L. J.
1921. EXPERIMENTS IN FIELD PLOT TECHNIC FOR THE PRELIMINARY DETERMINATION OF COMPARATIVE YIELDS IN SMALL GRAINS. Mo. Agr. Expt. Sta. Research Bull. 49, 78 pp., illus.

(19) ———
1929. EXPERIMENTAL ERROR IN FIELD-PLOT TESTS. Internatl. Cong. Plant Sci., Ithaca, N. Y. Proc. v. 1, pp. 107–127.

(20) STRINGFIELD, G. H.
1928. TYPES OF FIELD AND PLAT IN CROP TESTS. Jour. Amer. Soc. Agron. 20: 1073–1096, illus.

(21) SUMMERBY, R.
1923. REPLICATION IN RELATION TO ACCURACY IN COMPARATIVE CROP TESTS. Jour. Amer. Soc. Agron. 15: 192–199, illus.

(22) TEDIN, O.
1931. THE INFLUENCE OF SYSTEMATIC PLOT ARRANGEMENT UPON THE ESTIMATE OF ERROR IN FIELD EXPERIMENTS. Jour. Agr. Sci. [England] 21: [191]–208, illus.

(23) TIPPETT, L. H. C.
1931. THE METHODS OF STATISTICS; AN INTRODUCTION MAINLY FOR WORKERS IN THE BIOLOGICAL SCIENCES. 222 pp., illus. London.

(24) WOOD, T. B., and STRATTON, F. J. M.
1910. THE INTERPRETATION OF EXPERIMENTAL RESULTS. Jour. Agr. Sci. [England] 3: [417]–440, illus.

(25) YULE, G. U.
1929. AN INTRODUCTION TO THE THEORY OF STATISTICS. Ed. 9, rev., 424 pp., illus. London.

MORPHOLOGY AND BIOLOGY OF THE WHEAT JOINTWORM GALL[1]

By W. J. PHILLIPS, *entomologist*, and F. F. DICKE, *assistant entomologist, Division of Cereal and Forage Insect, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture*[2]

INTRODUCTION

The study of the morphology of wheat-stem galls caused by the wheat jointworm, *Harmolita tritici* (Fitch), was begun by the senior writer in 1927 at Charlottesville, Va., but it was incidental to the investigation of the insect itself and the measures for its control. During the winter of 1932–33, however, it appeared that with more material available facts of definite economic importance might be revealed. In the spring of 1933, therefore, a large number of galls, collected during the period of development of the insect from hatching to maturity of the larva, were preserved at Charlottesville. During the previous winter, arrangements had been made with the laboratories located at Wichita, Kans., and Lafayette, Ind., to preserve wheat stems in their various stages of growth. Since then a comparative study of the plants from the three localities relative to their susceptibility to jointworm attack, has been made.

As late as 1904 F. M. Webster stated that *Harmolita tritici* was not known to occur west of the Mississippi River. Since that time it has been found in the Wheat Belt as far west as central Missouri and also in Utah, California, and Oregon. The results of this investigation may explain why the wheat jointworm does not occur in Kansas and how its ravages may be materially reduced in the Eastern and Central States.

HISTORICAL RÉSUMÉ

A large amount of work, mostly in Europe, has been done on the morphology and biology of insect galls. Anyone interested in this subject should consult the bibliographies given by Kinsey (*6*)[3] and by Ross (*9*). Neither bibliography is complete, but each supplements the other. Ross does not list North American works. His book is very recent, however, and is of more general interest inasmuch as it covers the whole field of gall-forming organisms.

In reviewing the literature on gall-forming insects, one is struck by the small amount of work that has been done on the chalcids. This may be due to the fact that, so far as known, all chalcid galls are small and inconspicuous, and perhaps are found mostly on the grasses.

Only three published articles on the morphology and biology of *Harmolita* galls have been found. One paper (*8*) deals with galls on *Ficus* spp. and *Cattleya* sp.[4] and the other two (*3*, *5*) are on the terminal and axillary bud galls of *Agropyron* spp. These papers are of little

[1] Received for publication Dec. 3, 1934; issued April 1935.

[2] The authors acknowledge their indebtedness to W. H. Larrimer, under whose general supervision the work was done, for encouragement and helpful suggestions; to W. R. Walton for criticism of the manuscript; and to Ivey F. Lewis, of the Miller School of Biology, University of Virginia, and Ernst Artschwager, of the Bureau of Plant Industry, U. S. Department of Agriculture, for valuable suggestions on technic.

[3] Reference is made by number (italic) to Literature Cited, p. 385.

[4] These gall makers, in all probability, do not belong to the genus *Harmolita*.

interest in the present study, since they involve different species and different parts of the plants, and the plant responses are very different. Besides, the wild grasses involved continue growing throughout the season, as is largely true of the bud galls on them, whereas the wheat jointworm galls mature slightly in advance of the wheat plant.

METHODS AND PROCEDURE

In order to make a complete study of the morphology and biology of the galls produced by *Harmolita tritici*, tissues were preserved daily from the moment the eggs were deposited until the larvae became full grown. To accomplish this, wheat plants were transplanted to 12-inch flowerpots, and each plant was labeled and enclosed within a 10-inch glass cylinder, the upper end of which was covered with cheesecloth (fig. 1, *A*). From 30 to 40 females were then introduced into each cage, and the place of oviposition on the culms and the approximate number of eggs deposited were recorded. No female was allowed to remain in a cage longer than 8 hours, frequently half that period being sufficient for infestation of all susceptible culms. Plants in the field also were covered with cylinders in the same manner, and oviposition was recorded. These field plants were used principally for studying the later instars of the larvae and the older gall tissue.

Fixing solutions of several kinds were used, but the formalin-acetic-alcohol mixture gave the best results. The tissues were demineralized in 10-percent hydrofluoric acid for 3 days, dehydrated, and cleared according to Zirkle's (*13*) butyl-alcohol method. Hance's (*4*) paraffin-rubber mixture was used for embedding during the winter of 1933–34. In previous work the old method with xylol and ordinary paraffin had been followed, but the butyl alcohol and paraffin-rubber combination was found to be superior. Sections were cut to a thickness of 14μ and stained in iron-alum haematoxylin. Gentian violet, acid fuchsin, and orange G were used as counter stains. The violet was found to be a more satisfactory counter stain for the older tissues.

OVIPOSITION

DEPOSITION OF THE EGG

Harmolita tritici oviposits in the rapidly growing culms of wheat after these have reached a certain critical stage of development. When the complete mechanism of oviposition is thoroughly understood it becomes at once one of the most interesting and, perhaps, most important phases of the whole subject of wheat jointworm gall formation. It will, therefore, be treated at some length.

The female locates a suitable point for oviposition by walking slowly up and down the wheat culm, tapping its surface continually with her antennae as she moves along. This process may occupy considerable time even when a culm in the proper stage of growth is under examination. The place selected for oviposition is at the upper side of a node, in tissue that will later form the internode. The ovipositor (fig. 1, *B*) is inserted slightly below the meristematic region, at the union of a leaf base with the stem (fig. 2, *B* and *C*).

It will be noted from figure 1, *C* and *D*, that there is as yet no visible evidence of the node at the point of oviposition, as the internode below has not lengthened sufficiently to elevate it into view.

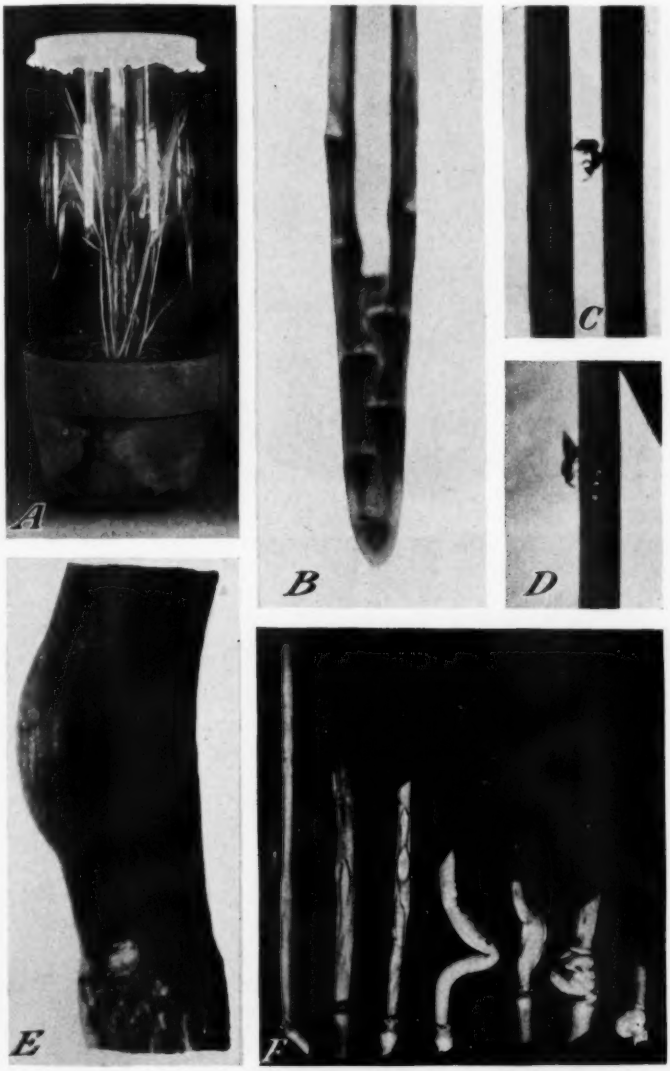

FIGURE 1.—*A*, Breeding cage; *B*, the spearhead of the ovipositor lance, × 628; *C*, female *Harmolita tritici* in the first position for oviposition, × 2; *D*, female in the final position and in the act of oviposition, × 2; *E*, internode that was too old and tough at the time of oviposition to respond properly to the stimuli incident to the presence of the larvae, and the expanding gall tissue ruptured the epidermis, expelling a larva (p. 367), × 6; *F*, different types of galls that may be found in any wheat field infested with *H. tritici*, the long gall at the left is the type most frequently found, and the two warty galls at the right are the kind that develop from the type illustrated at *E* in which the larvae rarely mature; almost natural size.

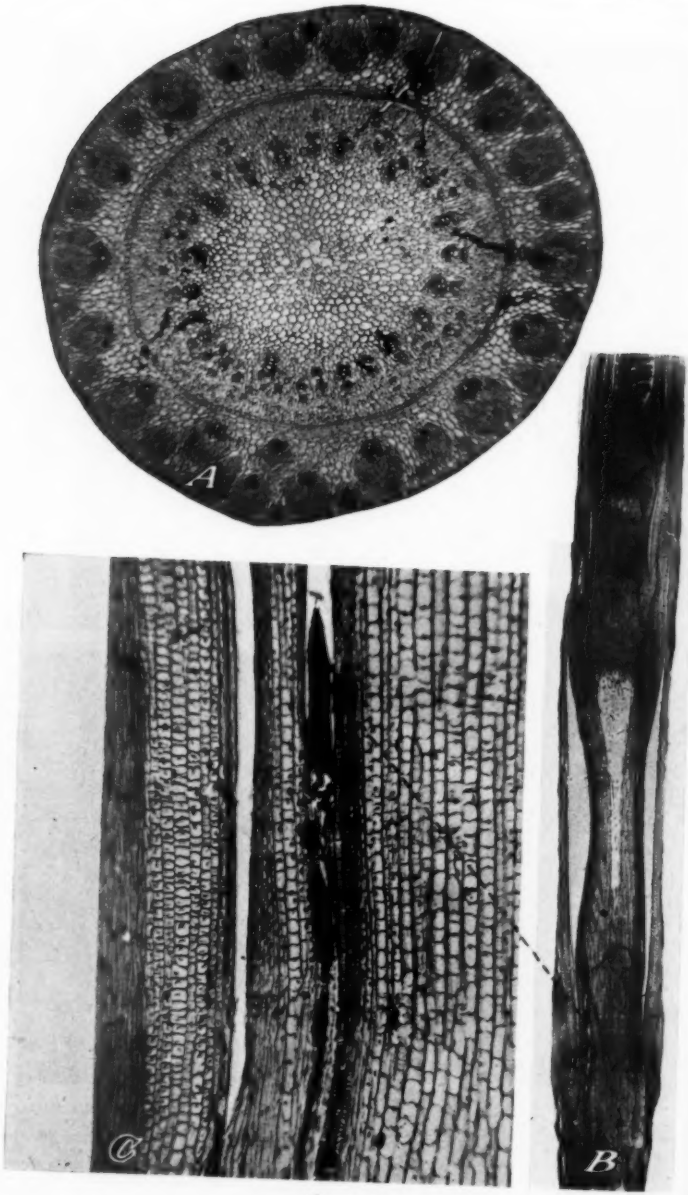

FIGURE 2

(For explanatory legend see opposite page)

That is, the node at the oviposition point is covered by the sheath of the next lower leaf. The only way one can definitely locate such a node without removing the sheath is by pressing the stem between the fingers. Occasionally females do attempt oviposition at an exposed node where the tissue is older and harder, but such nodes are difficult to pierce, and larvae do not mature in this older tissue.

After selecting the point for oviposition, the female, with her head directed down the stem, lowers the abdomen to the surface of the culm, and, at the same time, she brings the tip of it forward until the abdomen is at right angles to the thorax (fig. 1, *C*). The ovipositor is pointed into the tissues and the abdomen is elevated to almost a vertical position. The ovipositor is used very much as a gimlet to bore a hole through the leaf sheath (fig. 2, *A*) into the tender tissues of the culm, slightly below the meristem of the internode, until a vascular bundle is reached within the culm. By referring to figure 3, *C*, it will be seen that the phloem lies beside and external to the xylem in a vascular bundle of the collateral type. The ovipositor passes through the phloem until it reaches the xylem tubes, the hardest elements of the internal tissues at this point, then it turns upward, following the tubes (fig. 2, *B*) until the hole made by the ovipositor is of sufficient depth. At this time the insect is in the position shown in figure 1, *D*. The female remains in this position for several minutes in order to allow the egg to pass down the oviduct and into the tissues of the plant. The ovipositor is then partially withdrawn and thrust back into the tissues in a different direction (fig. 2, *A*) in search of another vascular bundle, where an additional egg is deposited. This latter process may be repeated 5 or 6 times, resulting in the deposition of as many eggs through one external opening. It may be seen (fig. 2, *A*) that all the eggs cannot be placed in the same horizontal plane, even though the same external opening is used. Those eggs placed in the vascular bundles immediately under the body of the female will be highest in the internode as they are placed at the farthest point attainable, and those deposited in the bundles to her right or left will be lower. The insect doubtless finds the epidermis difficult to pierce with the ovipositor and probably saves a great deal of energy by depositing several eggs through a single opening.

After oviposition is completed at the first external puncture, the female frequently moves around the stem in the same horizontal plane (fig. 2, *A*) and bores a second hole to insert more eggs. Occasionally as many as 16 eggs are deposited in the meristematic tissue immediately above a node (fig. 3, *A*). Figure 3, *B*, shows a highly magnified vascular bundle with an egg in place and almost against the xylem tube, and figure 3, *C*, shows a normal bundle of the same age. The average in nature, as shown by dissection of many mature galls, is about 6 or 7 larvae to the infested internode.

EXPLANATORY LEGEND FOR FIGURE 2

A, Cross section of a wheat culm, at the top of a node, through an oviposition puncture 1 day old. Note how the channel branches towards the vascular bundles; below this, and farther around the stem, may be seen traces of other channels emanating from another puncture that does not show in this section. On the opposite side of the stem may be seen still fainter traces of channels from another oviposition puncture that is not shown; × 25. *B*, A longitudinal section of the three upper nodes and the embryonic head of a wheat culm. All the three nodes and internodes together measured about one-half inch. The dotted line indicates the position of the day-old egg in the meristem, just above the node; × 7.8. *C*, The egg and the surrounding meristematic tissue shown in *B*, but more highly magnified; note how closely the oviposition channel follows the xylem tubes and that the egg lies in the phloem with one side almost against the xylem tubes; × 58.

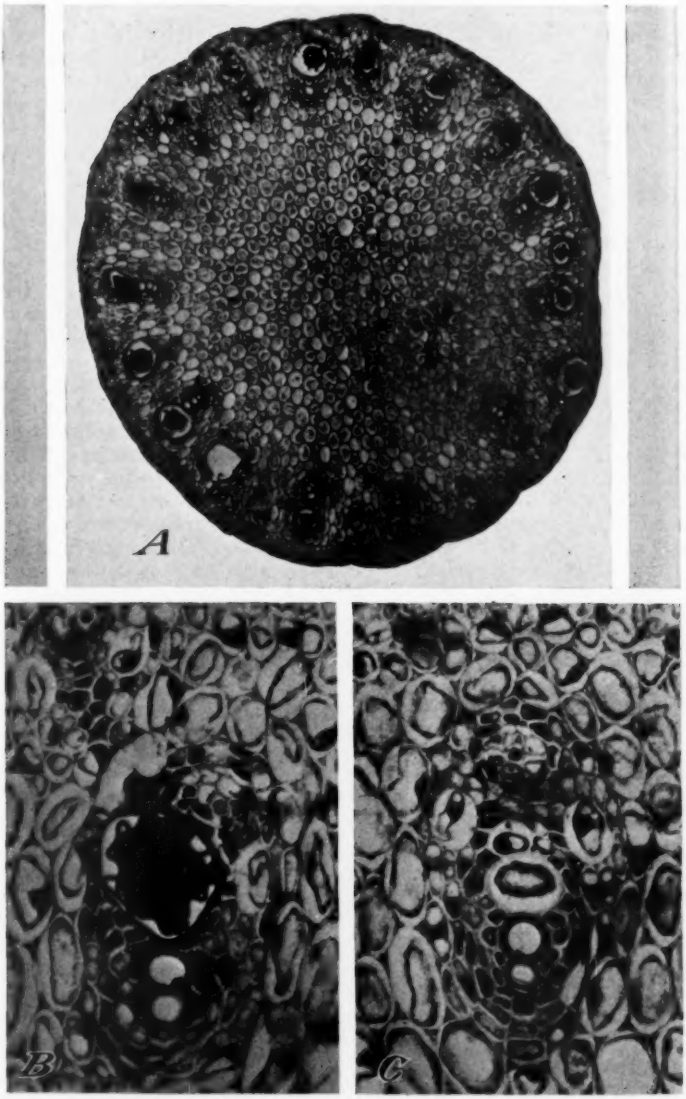

FIGURE 3.—*A*, Cross section of internode containing 16 day-old eggs, × 64. All the eggs are in the phloem of the vascular bundle and there are only three large bundles that do not contain eggs. *B*, Vascular bundle 1 hour after oviposition, × 330. *C*, Normal vascular bundle from the same section as *B*, × 330.

The eggs are always found in the phloem of the vascular bundle (fig. 3, *A* and *B*), or in the parenchyma immediately adjoining it. Numerous galls have been sectioned, involving the cutting through of many eggs, and, with perhaps one exception, the female has never been found to miss the mark; and in this exceptional case the egg was located very close to the phloem. Although the eggs are usually placed in the large bundles, an egg may occasionally be found in one of the small vascular bundles near the epidermis; however, even here the egg is properly placed in the phloem.

The writers have never found a record in literature to indicate that any female gall-making insect selects the phloem for the placement of the egg. This, of course, is the most favorable location for the placement of the eggs, since the prospective larvae would be surrounded by rich food material immediately upon hatching. Other gall-making insects select various parts of the host plant, the eggs of some being placed even on the surface of leaves, the gall tissue growing up around them from all sides. A number place their eggs in the cambium layers and others under the growing points of buds. In general it appears that the vascular system of gall tissue is simply a modified extension of the regular vascular system of the neighboring normal tissue, whereas in the case of the wheat jointworm gall the normal vascular system of the internode involved, as later described, is almost entirely destroyed (figs. 4, *B*, *C*, and 11, *A*, *B*).

The fact that the eggs are precisely placed in the phloem makes oviposition a tedious operation. If the female could puncture the meristem at any point without regard to its age and texture and could insert the eggs in a haphazard manner, the process would be greatly simplified. It is necessary for her to probe the tissues carefully to locate the vascular bundle in which to place each egg. Since only one egg is placed in the phloem of any bundle, a fresh bundle must be sought for the deposition of each individual egg. The larvae thus find themselves so placed as to intercept the rich sap flowing in the main conduction channels carrying elaborated foods and, therefore, have the first call on the resources of the plant.

FACTORS INFLUENCING OVIPOSITION AND THEIR RELATIONSHIP TO CONTROL MEASURES

The most important factors influencing oviposition are temperature, the size of the host plant, and the structure of the meristematic regions of the culms where the eggs are to be placed.

Oviposition occurs very sparingly at temperatures below 70° F. and is most active at or a little above 80°. Regardless of temperature, adults are inactive at night and during stormy weather. They are rather frail creatures and heavy rain and wind storms are very disastrous to them. Under the most favorable circumstances a female may deposit the majority of her eggs and die at the end of 2 days. Apparently no eggs develop after the female emerges. Cold, cloudy weather may prolong the lives of the adults for an additional 3 or 4 days.

The portion of the culm usually most attractive for oviposition is only about one-fourth inch in length, or, if there should be two more nodes above this, all three together at this time would measure about a half inch (fig. 2, *B*), and it is rare that on any one culm there will

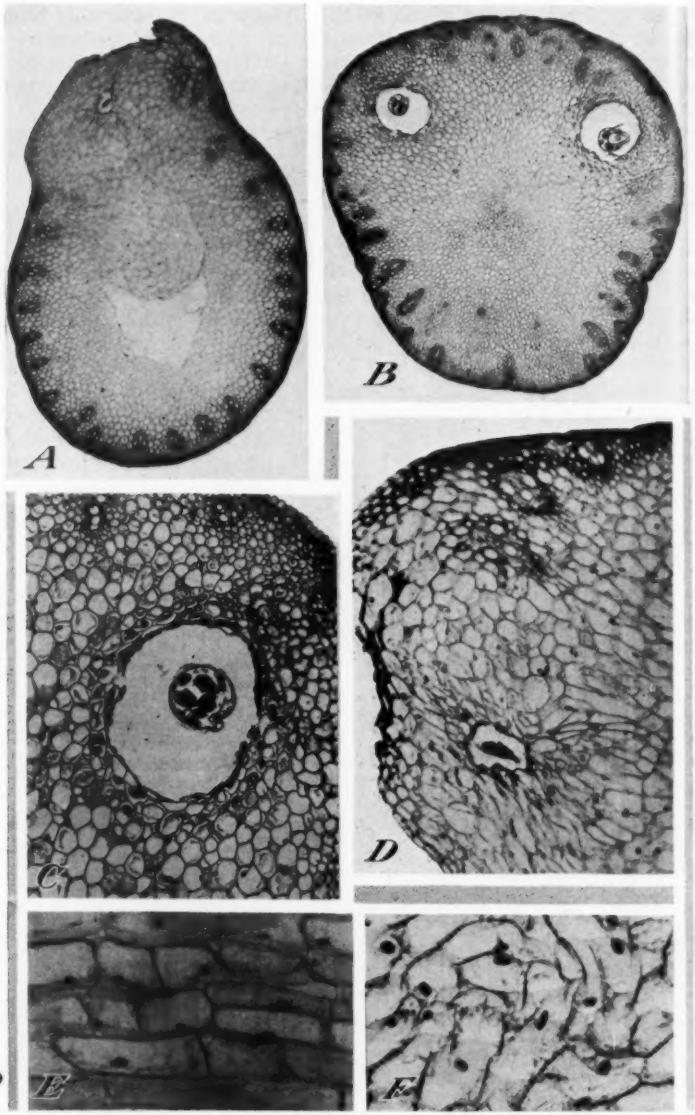

FIGURE 4.—*A* and *B* are transverse sections of two galls from the same culm during the second larval period. The eggs for each gall were deposited the same day, and the tissues were preserved in a fixing solution when at the same age. The gall from which *B* was cut was the internode immediately above *A*, and therefore the younger and more tender internode. Note that the larvae and the gall tissue have developed normally in *B*. The tissue in *A* was too old to respond to the stimulus incident to the presence of the larvae, and normal gall tissue did not develop; *A*, × 18.7, *B*, × 23.6. *C*, Normal food tissue surrounding normal larvae shown at the upper left in *B*, × 64. *D*, A portion of *A* at higher magnification; all larvae are dead in this internode and no rich food tissue surrounds them; the larvae starved and died in consequence, × 44. *E*, Normal parenchymatous tissue outside the gall area of the second larval instar, × 138. *F*, Section illustrating loss of polarity in gall tissue of the second larval instar, × 150.

be on one day more than one attractive node. Regardless of favorable weather conditions the females will ordinarily not oviposit in any but attractive material, but frequently a female will work industriously in an effort to oviposit in a second internode and may succeed in placing several eggs. Larvae hatching in such positions cause warty or irregular deformations, as shown in the two very short galls in figure 1, *F*. Rarely do such larvae reach maturity because the tissues in these locations are too old to respond to the stimuli incident to their presence, with the result that insufficient food material to nourish them is deposited in the cells immediately surrounding the larvae (fig. 4, *A* and *D*). Occasionally the tissues are so hard that the pressure of the enlarging cells within ruptures the epidermis and expels the larva from the plant, as shown in figure 1, *E*. Typical galls of *Harmolita tritici* are shown in figure 1, *F*. The longer galls (at the left) are the forms most commonly observed.

This very important fact was strikingly illustrated in one of the field observation cages in 1933. Females oviposited in two internodes of a culm on the same day. When the larvae were in the second instar, as determined by dissections of other plants of the same cage, the gall tissues at the two infested internodes of this culm were killed in fixing solutions at the same time. These two galls were embedded and sectioned in January 1934. Figure 4, *A*, *B*, *C*, and *D*, shows the gall tissue in the higher internode to be perfectly normal in every way, and the developing larvae are normal. The larvae in the lower, older internode are dead and the gall tissue is not normal. The tissues at this internode were too old and hard when the eggs hatched to respond in the usual manner to the stimuli incident to the presence of the larvae, with the result that sufficient food materials to nourish them were not deposited in the immediately surrounding cells. Thus, the larvae starved not because the plant was not manufacturing sufficient nourishment to supply them, for the larvae located in the tender meristematic tissues at the internode immediately above were then being properly nourished, but because the tissues had become too tough and hard to respond to the stimuli.

Wheat culms may be found having several internodes infested by the jointworm. This is caused either by the emergence of females over several days, or by the survival of individuals for 5 or 6 days. A period of 1 day's growth may serve to render a lower internode unsuitable for oviposition. In the meantime the internode immediately above may become suitable for oviposition. It would be misleading, therefore, to say that any particular internode of the wheat culm is preferred by the female for oviposition. Any internode may be selected in which the meristem is in a stage of development acceptable for oviposition.

It is obvious from the foregoing statements that a delicate balance exists between the period of emergence of *Harmolita tritici* and the development of the meristematic regions of the internodes in the host plant. Plants that are small and medium sized at the time of wheat jointworm emergence are therefore more likely to have their meristematic regions in the stage attractive for oviposition. In a large stem the leaf sheath is thicker, and the vascular bundles lie deeper in the culm, making it more difficult for the insect to reach the vascular tissues in which to place her eggs. It should be practical, therefore, to hasten the growth of wheat in the spring by fertili-

zation, or otherwise, so that the plants would be too far advanced in growth to be in a condition attractive for oviposition when the adults of the jointworm emerge. Failing in that, the advanced stage of growth thus induced would oblige the adults to oviposit so high up in the stem that the galls would be removed with the straw by the reaper at harvest and thus their occupants would no longer be a threat to the succeeding crop. Under average conditions the majority of the galls are so near the ground that they are left in the stubble at harvest.

COMPARISON OF THE MERISTEMATIC REGIONS OF WHEAT PRODUCED IN KANSAS, INDIANA, AND VIRGINIA AS RELATED TO OVIPOSITION

After it was learned that the condition of the meristematic regions of the internodes governed oviposition, it was decided to compare wheat plants from Kansas, where *Harmolita tritici* does not occur, with plants from Indiana and Virginia, where *Harmolita tritici* has caused considerable injury to the wheat crop.

Arrangements were therefore made with the Federal entomological laboratories at Wichita, Kans., and Lafayette, Ind., to preserve whole plants in formalin-alcohol-acetic fixative solution. Wheat plants grown in Virginia were preserved in a similar manner. At 2-day intervals during a period of 18 days, starting a few days before emergence of *Harmolita tritici* adults and ending after the approximate hatching period for the eggs, 50 plants were collected from a field of a variety commonly grown in each locality, and preserved. In Kansas, the time of collection and preservation of stems was based on the emergence of a closely related species, *H. grandis* form *grandis*, which occurs in Indiana and Virginia as well as in Kansas.

The wheat plants from the several localities were examined at the same time at the Charlottesville laboratory, and it seemed, from external appearances, that there were nodes in the proper stage of development for oviposition in all the samples, although the Kansas plants were below the average height and diameter of those from Virginia and Indiana. In this respect it appeared that the Kansas plants might be the more suitable for oviposition. A difference between them and the plants of more eastern origin was found in the firmness of the meristem. This part in the Kansas plants was so firm that it could scarcely be crushed between the forefinger and thumb, whereas in the Indiana and Virginia plants the meristem was spongy and crushed under slight pressure.

Present knowledge of the responses in the tissues of the meristematic regions at the nodes of the wheat plant during gall formation plainly shows that tissues such as those in the Kansas wheat are in a hardened condition and unsuitable for oviposition at the time of emergence of *Harmolita tritici*. Moreover, should the insect succeed in ovipositing in such culms the hatching larvae, in all probability, would not survive.

It is not known at present whether this condition of the meristem in the Kansas plant is due to a varietal or a climatic difference. The main point of interest and importance is that this characteristic undoubtedly makes wheat plants grown in the winter wheat belt of Kansas either unacceptable to *Harmolita tritici* for oviposition, or unsuitable for larval development, or, perhaps, both. If this be true of the Kansas area it is probably true of other sections in the winter wheat belt of the United States where *Harmolita tritici* does not occur.

EFFECT OF OVIPOSITION ON THE PLANT AND SPACING OF THE LARVAE IN THE INTERNODE

The effect of oviposition on the plant tissue is almost negligible, insofar as its normal functions are concerned. The oviposition punctures heal, and scar tissue is found in the path of the ovipositor (fig. 5, *E*). The tissue heals around the eggs, and at the end of a few days the latter appear as inert foreign material embedded in normal tissue (fig. 6, *A* and *D*). Many of the cells immediately above the egg, however, lose their polarity, owing to the disturbance at the time of oviposition (fig. 6, *A*). If the eggs are dissected from the stem just before hatching time there does appear to be the beginning of a gall, although in reality this is simply a cavity in the tissues made by the ovipositor, somewhat larger than the egg, which has completely healed over.

Apparently the normal amount of cell division and other activities proceed as usual after the eggs are placed in the tissues. A few of the cells in the meristematic regions at this time show two nuclei (fig. 6, *B* and *C*). As the cells of the meristematic region divide and increase in length, the eggs, not being deposited in exactly the same horizontal plane, are carried upward various distances in the tissues, and when the larvae are full grown there may be from 1 to 2 inches difference in height between the lowest and highest placed larvae in the same internode (fig. 1, *F*).

SEASONAL HISTORY OF THE GALL

HATCHING AND FEEDING OF FIRST-INSTAR LARVAE

The egg is placed in the tissues with its petiole extending down the stem, and when it hatches the larva also has its head pointing down the stem. The larva apparently ruptures the shell with its delicate mandibles at the union of the petiole and the main body of the egg. The shell is not cast off at once, and the larva feeds head downward, at the point of rupture. From dissections made at this time, it appears that the young larva is bathed in plant sap. It is not definitely known whether some of the surrounding cells are pierced by the mandibles of the first-instar larva, in order to maintain a constant flow of sap, or whether this activity is unnecessary at that time. There is a well-developed digestive tract, even in the first-instar larva, and the greenish, chorophyll-tinted food in this tract is plainly visible through the body wall. Serial section mounts show that there is no perceptible accumulation of deeply stained matter around the margin of the larval chamber, such as is always present in later instars, indicating that it may not be necessary in the first instar for the larva to puncture the cells in order to obtain sufficient nourishment.

BEGINNING OF GALL FORMATION

By the time the jointworm egg hatches, an uninfested internode of the same stage of development as the infested one has ceased cell division, and normal length and diameter are very soon attained by growth of the cells. When the larva starts feeding, the cells in the immediate vicinity suddenly become very active and increase markedly in size, the nuclei and nucleoli enlarge greatly, the protoplasm becomes very dense, and the walls are thin (fig. 7, *A*). Just beyond this region, which is only 2 or 3 cells deep, the cells begin to divide

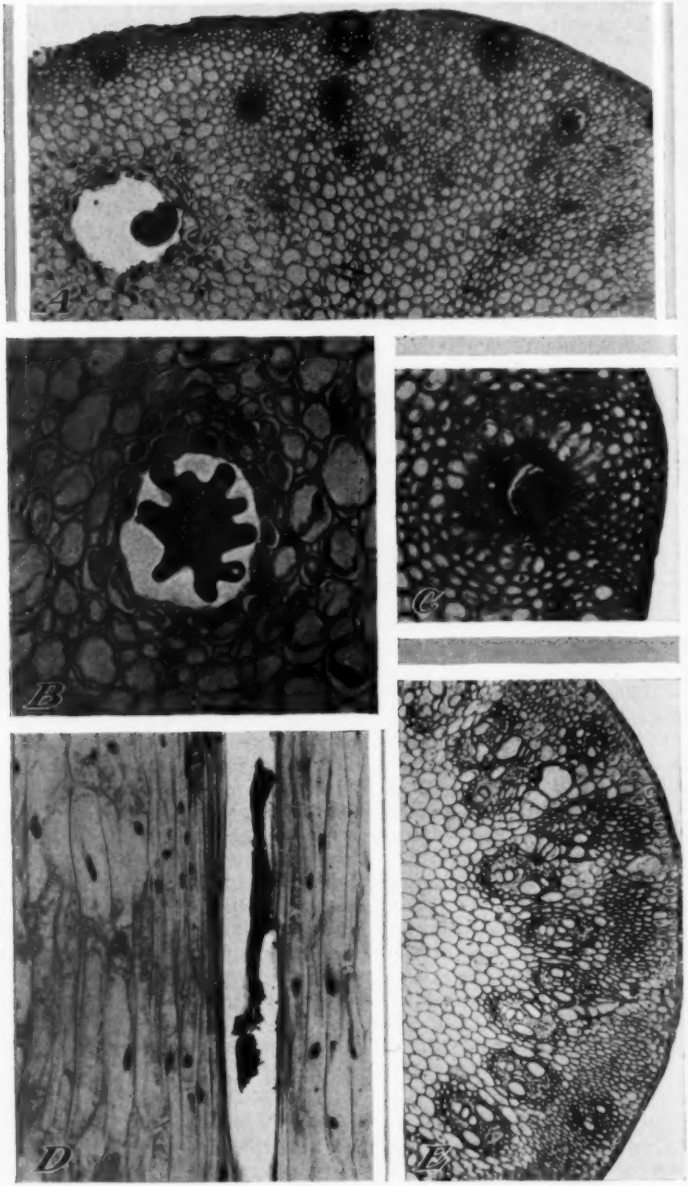

FIGURE 5

(For explanatory legend see opposite page)

(mitotically) rapidly. This change occurs in the vicinity of all larvae, regardless of their position with relation to the meristem. For example, figure 8 shows a larva located a considerable distance up the internode, well out of the active meristematic region, yet cell division is proceeding as rapidly as in the vicinity of larvae located much nearer the node.

Figure 9, *C*, shows recently divided cells lying just outside the area of enlarged cells but immediately surrounding the larva. Note in this figure that these divisions may take place in any plane and that this results in cells of various shapes. Thus, the lack of normal polarity of some cells caused by oviposition (fig. 6, *A*), in addition to the very rapid growth of the cells immediately surrounding the larva, causes the cells in the gall tissue to become irregular both in shape and in arrangement. Figure 9, *B*, shows normal tissue located outside the gall area. Note how regular these cells appear. Figure 9, *A*, shows normal tissue at time of oviposition. Figure 8, *A* and *B*, shows a newly hatched larva, well up the internode, that has induced active cell division. Under normal conditions no further cell division would take place in this region. In other words, the immediate results of hatching and feeding of the young larvae are hypertrophy and hyperplasia, provided the tissues are still plastic enough to respond to the stimuli. Where the tissues are too old, the result is simply hypertrophy, and the larva starves and eventually dies (fig. 4, *A* and *D*), since the tissues are then unable to deposit sufficient food materials to nourish it. It is a fact well known to students of gall tissues, that a gall-producing larva cannot stimulate old tissues into activity.

ACTIVITY DURING THE SECOND INSTAR

Before the second instar is reached, cell division ceases and all later growth is by simple hypertrophy. The cells in the immediate vicinity of the second-instar larva become very much larger than in the case of the preceding instar (fig. 7, *A* and *B*), and the cells associated with succeeding instars are progressively larger (fig. 7, *C*). Figure 10, *A*, shows some of the cells in the food-tissue zone of the third instar more highly magnified to illustrate the density of the protoplasm. By examining figure 10, *E*, the great contrast between the gall tissue and normal tissue will be seen. The nuclei and protoplasm have the same general appearance, except that the nuclei and nucleoli are very much larger and the nucleoli are of irregular shapes in later instars (fig. 10, *B*, *C*, *D*). These cells are what the European writers on insect galls have called "food tissue." There is no definite number of layers of such cells about a larva nor are they arranged in any particular direction as in some of the cynipid galls; they have lost their normal arrangement and point in various directions (fig. 4, *F*), although the general trend of the long axes of the cells is vertical (fig. 7, *A* and *B*).

EXPLANATORY LEGEND FOR FIGURE 5

A, Part of a transverse section that contained a normal second-instar larva and an unhatched egg, × 75. Egg may be seen in the upper right corner. *B*, Unhatched egg highly magnified (× 400), showing that the egg is surrounded by parenchymatous tissue, whereas the larva shown in *A* has the normal gall tissue developed about it. *C*, Unhatched egg in part of a transverse section in which normal third-instar larvae were also present. Note that the parenchymatous tissue surrounding this unhatched egg is now thick-walled and lignified, × 112. *D*, Part of a longitudinal section containing a dead second-instar larva. Note that the cells surrounding the larva have changed from the normal food tissue cells to the parenchymatous type, × 100. *E*, Scar tissue developed in the path of the ovipositor, 9 days after oviposition. No gall tissue has developed here, × 64.

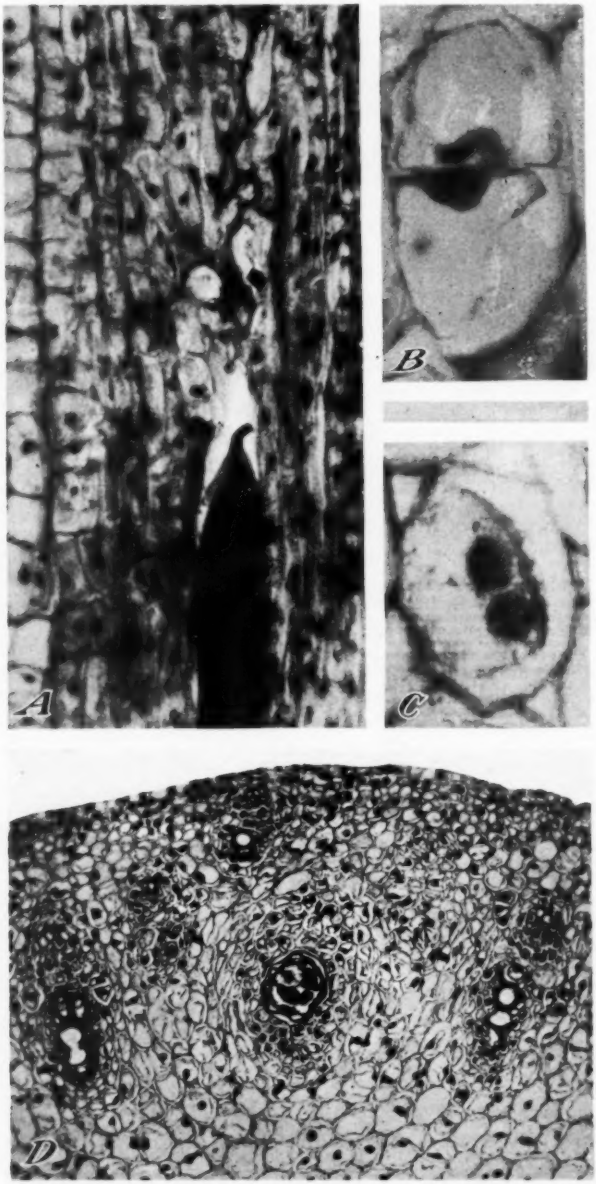

FIGURE 6.—*A*, Longitudinal section through an egg 7 days old. Note that the cells above the egg have lost their normal polarity, × 180. *B* and *C*, Cell with two nuclei from tissue containing eggs 9 days old; *B*, × 1,030; *C*, × 790. *D*, Cross section through an egg 9 days old. Note that the plant cells have closed the cavity about the egg, and the vascular bundle in which it was originally placed no is longer recognizable, × 115.

Every vascular bundle that contains a larva is entirely disintegrated. In case the internode contains as many as 15 or 16 larvae, the vascular tissue will have almost entirely disappeared. The vascular tissue that may be found in such gall tissue consists of a few irregular bundles, all of which may be pushed to one side of the stem (fig. 11, *A* and *B*). These bundles are not parts of vascular tissue originating in the new gall tissue. Therefore, practically all food materials that pass through this area must apparently be largely transferred through the gall-tissue cells, as the normal vascular system no longer exists. Naturally this derangement in the vascular system is a great hindrance to the plant and a serious drain upon its resources, as is indicated by the fact that the yield from infested plants is much less than from normal plants of similar size.

As practically all of the vascular bundles are destroyed in the infested portions of the internodes, the culms become very weak at these points, and consequently a windstorm or heavy rain will cause the infested culms to fall or lodge. This will occur only during the first or second instar of the insect, since in the later instars the gall has a large amount of modified supporting tissue developing in it (fig. 12, *B*). Frequently the weight of the culm alone will cause the internode to loop (fig. 1, *F*) in the gall area where the vascular bundles have been destroyed. The heads and galls of fallen or lodged plants frequently escape the harvester. This permits the galls to remain as a source of infestation in the following season.

By the time the larvae have reached the second instar, the internode within the gall area is usually completely filled with thin-walled, parenchymatous gall tissue. This gall tissue arises mainly from the parenchyma, but partly from the phloem, during the first larval instar. No further cell division was observed during the later instars, the gall tissue increasing in volume by hypertrophy.

During the second instar the walls of the cells bordering the so-called "nutritive zone" (toward the center of the stem) begin to thicken and show pits (fig. 12, *A*). Lignification begins at this time. The condition of the nuclei and cytoplasm of these cells indicates that they are active, living cells.

As previously stated, when the larva breaks through the eggshell its head points down the stem. In the course of the second instar it reverses its position within the larval cell, and in all following instars its head points up the stem.

ACTIVITY DURING LATE INSTARS

As the larva grows older its mandibles become larger and more heavily chitinized and sclerotized with each molt, and it continues to puncture cells of the food tissue and to devour their contents. During the first and early second instars the main difference in the cells in the gall area is that the cells in the immediate vicinity of the larva are larger and more heavily charged with food material. All the gall cells are thin-walled at this time. The cavity occupied by the larva increases in dimensions through the destruction of the cells of the food tissue surrounding it, and the size of the larval cavity apparently keeps pace with the requirements of the growing larva. There is always a deep layer of rich food tissue about the larva regardless of the number of cells destroyed for food, and a constant supply of such

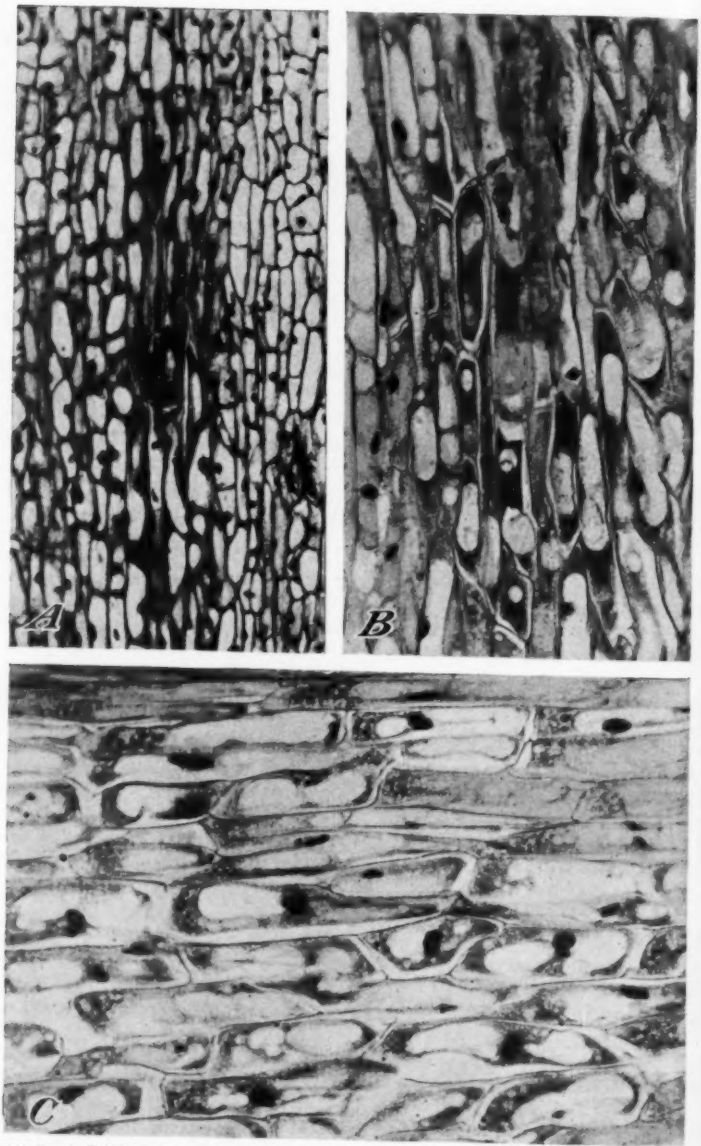

FIGURE 7.—*A*, Gall tissue near first-instar larva; *B*, gall tissue near second-instar larva; *C*, gall tissue near third-instar larva. These photomicrographs illustrate how the gall tissue increases in volume by hypertrophy; all × 100.

tissue is maintained by the enlargement of the cells already present. Cell division is not a factor in the maintenance of this food tissue.

As the larva and plant increase in age the condition of the cells shown in figure 12, *A*, becomes more pronounced (fig. 12, *C*). These

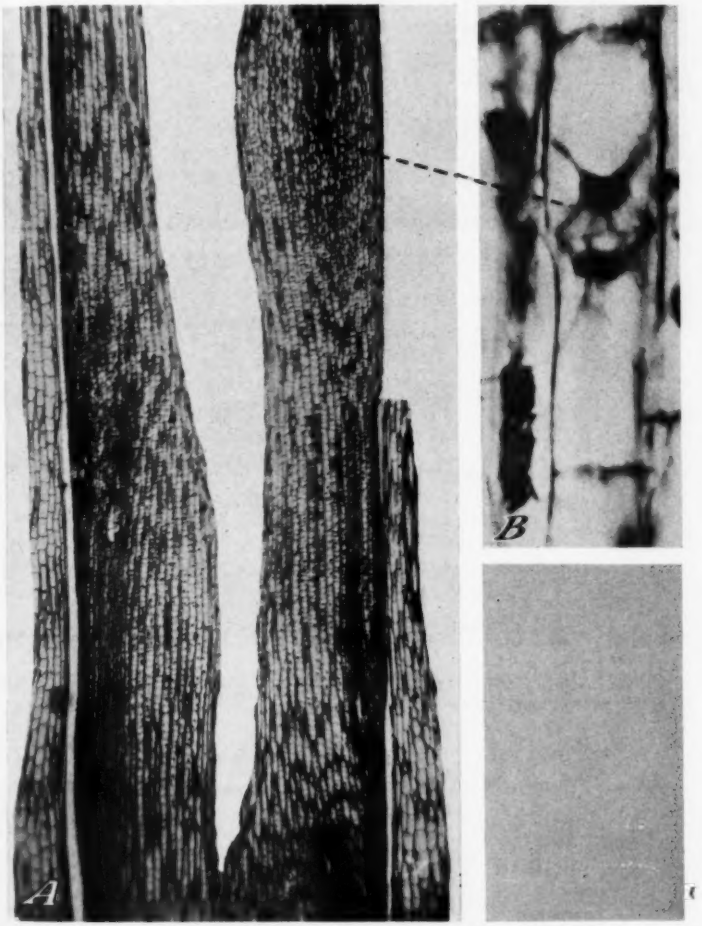

FIGURE 8.—*A*, Longitudinal section through an internode containing an early first-instar larva that was feeding but had not cast off the entire eggshell. The larva is the long, dark object on the right near the top. The wall of the internode is thicker at this point and the larva is quite a distance above the node. × 26.4. *B*, Mitotic cell division in the vicinity of the larva. This is the stage when hypertrophy and hyperplasia occur at the same time, × 850.

cells originate from the parenchymatous gall tissue, starting near the center of the stem and from those cells farthest from the larva (fig. 12, *B*). This lignifying tissue advances progressively toward the larval cavity until the food tissue is only 3 or 4 cells deep by the time the larva becomes full grown (fig. 11, *B*). Very shortly after the larva

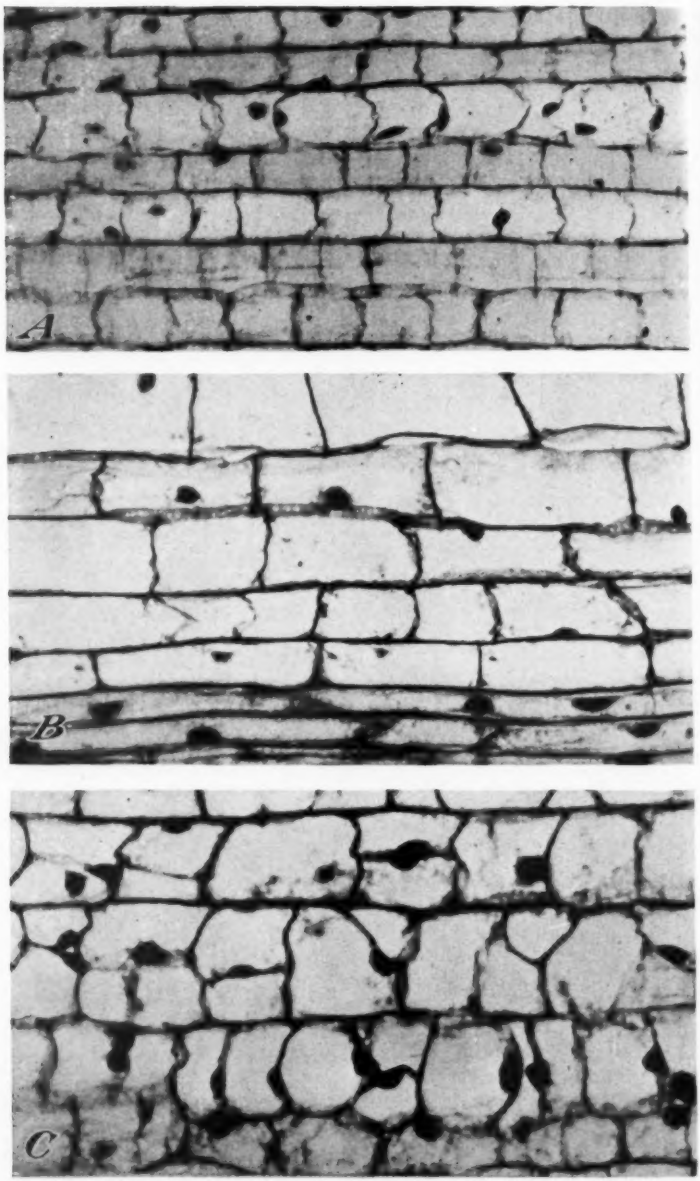

FIGURE 9.—*A*, Meristematic tissue at the time eggs are deposited, × 220; *B*, normal parenchymatous tissue outside the gall area in an internode with first-instar larvae, × 220; *C*, cells in the gall area near a first-instar larva, × 250.

stops feeding, all the tissue surrounding the larval chamber becomes lignified, and by the time the wheat is harvested this lignified gall tissue, with the exception of a thin layer around the larval chamber, has become as hard as white oak wood, instead of being in the normal condition of an uninfested stem as shown in figure 13.

The larva always maintains a very thin wall of cells between itself and the outside of the stem, and the full-grown larva is always found with the ventral surface of its body toward the outside of the stem. When, therefore, the adult casts off the pupal skin, the insect is ready to gnaw its way out through a very thin wall.

There is no indication that fecal matter is discharged into the larval cell or chamber at any time during growth. The larva changes to a prepupa in November and December, and the fecal matter is discharged at that time.

About a month elapses from the time the eggs are deposited until the larvae become full grown. The eggs hatch in about 12 days, which is almost half of this entire period. The first and second instars require about 5 days each, making 22 days from oviposition to the beginning of the third instar, and the remaining instars are completed in about 8 days.

It is interesting to note that this acceleration in development coincides with the rapid maturing of the wheat plant. If the last instars required as long as the first two, the larval period would then be carried beyond the maturity of the plant, and the larva would perish from lack of nourishment.

MICROCHEMICAL TESTS

As a matter of interest, a number of chemical tests were made of the gall tissue in serial sections on slides, according to Zimmermann's technic (*12*). Tests made for the presence of tannin and starch gave negative results; those for protein, cellulose, and lignin were positive. The contents of the cells in the food-tissue zone gave the positive test for protein, the cell walls in this zone gave the positive test for cellulose, and the thick-walled cells in the more mature gall tissue reacted to the lignin test.

ZONES IN GALL TISSUE

European and North American students of gall tissue state that several fundamental zones of tissue are found in insect galls, although they do not agree on the exact number. Cosens (*2, p. 354*) states that the cynipid galls have 3 or 4 zones, mostly 4; Küster (*7*) found 4; Weidel (*11*) and Ross (*9*) illustrated 4; Kinsey (*6, p. 40*) states there are, with a few exceptions, 5. According to Kinsey's interpretation, both Weidel's and Ross' illustrations may be construed as having 5 zones.

The wheat jointworm galls form within the internodes of the culms. Wheat stems do not have as many different kinds of tissue as do the leaves and twig tips of oaks; nor have the *Harmolita* galls as many tissue zones as the cynipid galls. Indeed, there are no sharply defined concentric tissue zones in the galls of *H. tritici* as have been described and illustrated for the cynipid galls. The gall tissues in the former arise partly from phloem, but mainly from the

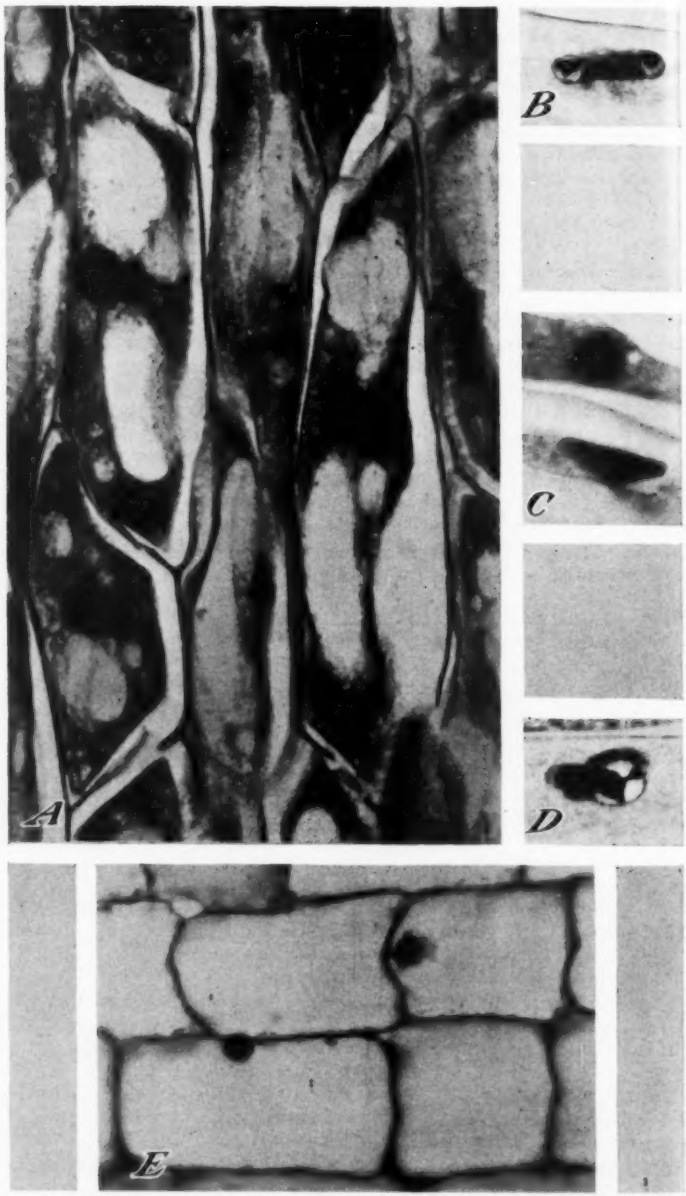

FIGURE 10.—*A*, Cells in the food tissue near the third-instar larva, highly magnified (× 275) to show the dense protoplasm and large nuclei. *B*, *C*, and *D* illustrate the peculiar shape of the nucleoli of some of the cells in the nutritive zone, × 580. *E*, Normal parenchymatous cells outside the gall area of third-instar larvae, × 580.

parenchyma, and fill the pith cavity. As is shown in the longest segment in figure 1, *F*, there is usually little external evidence of galls in wheat stems.

Ross' figure (*9*) of the gall on *Ficus* indicates that *Harmolita* galls on such plants are very similar to cynipid galls.

There are apparently no other galls mentioned in literature similar to jointworm galls on wheat stems. Most insect galls usually involve the leaves, petioles, veins, or leaf buds, whereas the wheat jointworm galls involve the main stem of the plant. There is no pith cavity in the gall area of the internode, the entire stem at this point being filled with gall tissue. There may be as much as 2 inches of the internode thus involved and, occasionally, in heavy infestations, 2 or 3 internodes may be infested. It is obvious, therefore, how seriously this gall tissue affects the normal functions of the plant.

The epidermis and sclerenchyma are changed very little during the process of gall formation, although these cells later become very thick walled and lignify (fig. 11, *A* and *B*). The parenchymatous cells immediately surrounding the larval chamber become heavily charged with very dense protoplasm and form what is known as the nutritive zone. There does not seem to be any definite number of cell layers to this zone, and there is no definite arrangement of these cells; in fact, a large number of the cells in the gall area lose their polarity as shown in figure 7, *A* and *B*, although the general trend is vertical. In the later larval instars these parenchymatous cells become thick walled, pitted (fig. 12, *A* and *B*), and lignified. The sclerenchymatous cells, just below the epidermis, and the epidermal cells also become thick walled and lignified. In the earlier instars of the insect, between the larval chamber and the epidermis are found the nutritive, the parenchymatous, and the sclerenchymatous zones. In other words, according to Kinsey's nomenclature, there are in the second larval instar 4 zones from the outside of the stem to the larval chamber, and 3 zones from the larval chamber to the center of the stem (fig. 11, *A*). When the gall tissue and the wheat plant are thoroughly mature there appear to be two zones of lignified tissue—a hard zone immediately surrounding the larva, and the remainder of the gall, which is very much harder.

THE GALL STIMULUS

Beyerinck (*1*) has stated that the initial stimulus of gall formation is a secretion from the poison glands introduced into the tissues with the ovipositor, and has figured a well-developed gall in which neither egg nor larva was found. Magnus (*8*) has stated that the secretion of the poison glands was without significance; that the start of gall formation was coincidental with the wounding of the tissue in oviposition; that the second impetus came with the feeding of the larva; and, further, that the gall tissue ceased development with the death of the larva. Cosens (*2, p. 371*) has stated that the larva secretes an enzyme that is the active agent in gall formation. Kinsey (*6, p. 37*) believes that the precise source and nature of the gall-producing stimulus are not known but thinks "it partakes of the nature of an enzyme or hormone produced by some particular insect structure."

Magnus (*8*) terms "Galligenes Organ" the short appendage at the posterior pole of the egg of the *Harmolita* from galls on *Ficus* spp. He

FIGURE 11.—*A*, Part of a transverse section through the gall tissue around a second-instar larva, illustrating the lack of vascular tissue, the food-tissue zone with the cells filled with rich food material, the thick-walled cells developing deeper in the gall tissue, and the manner in which the epidermal and the sclerenchymatous cells have been modified, × 50; *B*, part of a transverse section through the gall tissue at the time the larva is full grown, × 36. Note that practically all the tissue has lignified except the thin layer of food tissue around the larva. Note also that there are only a few small vascular bundles present.

suggests that a gall-forming irritant may pass out through it from the egg into the surrounding tissues. Magnus finds that several colloidal stains will readily pass into this appendage and diffuse to other parts of the egg. He also states that a large amount of gall tissue forms before the egg hatches. Ross (*9*) says that little is known about the gall-making irritant (Reizwirkung); that there are different kinds, some chemical, which arise mostly in the salivary glands and function as a kind of enzyme. Ross further states that there is a wound irritant (Wundreiz) that plays an important part in gall formation.

Apparently no gall-producing stimulus is introduced into the plant tissues at the time of oviposition by *Harmolita tritici*. This is clearly indicated by the fact that unhatched eggs about which no gall tissue had formed were found in tissues near second- and third-instar larvae (fig. 5, *A*, *B*, and *C*), whereas normal gall tissue had formed about the larvae. Furthermore, the condition of the cells in the vicinity of eggs 9 days old and of those in the vicinity of the eggs just mentioned appears to be the same (figs. 5, *B*; 6, *D*). As soon as the eggs hatch, gall tissue forms rapidly. Should the larva die for some cause after the gall tissue had formed about it, the food tissue ceases to function as such (fig. 5, *D*) and becomes modified parenchymatous tissue. It is obvious, therefore, that the presence of a living larva is necessary for the formation and continuance of gall tissue.

Smith (*10, pp. 177, 183, 184*) has shown that there are many substances, both alkaline and acid, that will cause proliferation of cells when introduced into the tissues of plants at the proper dilution. He says:

> From these experiments there can be no reasonable doubt, I think, that any soluble substance whatever, except a killing, a plasmolyzing or an oxygen-absorbing substance, if continually liberated in excess locally in tissues, would be competent to induce tumor formation.

He also says:

> * * * I think I have established my original hypothesis, viz, that dilute ammonia causes intumescences and have rendered it probable that ammonia liberated within the cell in small quantities by the imprisoned bacteria must be one of the causes of the excessive and abnormal cell proliferation in crowngall. Probably amin compounds also help to determine it. Since an acid and alcohol are likewise produced by the crowngall bacteria and this alcohol and this acid (as well as many other acids) in pure dilution and also in combination with ammonia caused galls or intumescences in my experiments, the acid (or acids), the alkalies, and the alcohol must, I believe, act together in producing the tumors, and *osmotically rather than chemically.* * * *
>
> To conclude, it would seem, therefore, that in local osmotic action (possibly in some stages chemical action also) of various substances (aldehyde, acetone, alcohol, acids, alkalies) thrown into cells and diffusing from them in various directions, as the result of the metabolism of a feeble intracellular parasite or symbiont together with the resultant countermovements of water and food supply we have, in crowngall at least and presumptively also in animal neoplasms, the explanation of tumor growth—that is, of that extensive multiplication of cells in opposition to physiological control which has so long puzzled pathologists and all students of overgrowths.

If the byproducts of metabolism of the crowngall organism, and various acids and alkalies, can cause tumor growths in plants, there would seem to be no valid reason why the byproducts of metabolism of larvae alone, when continuously liberated in plant tissues, would not produce galls. It also seems probable that irritation resulting from the larvae puncturing the cells of the food tissue might be a contributing cause.

128001—35——7

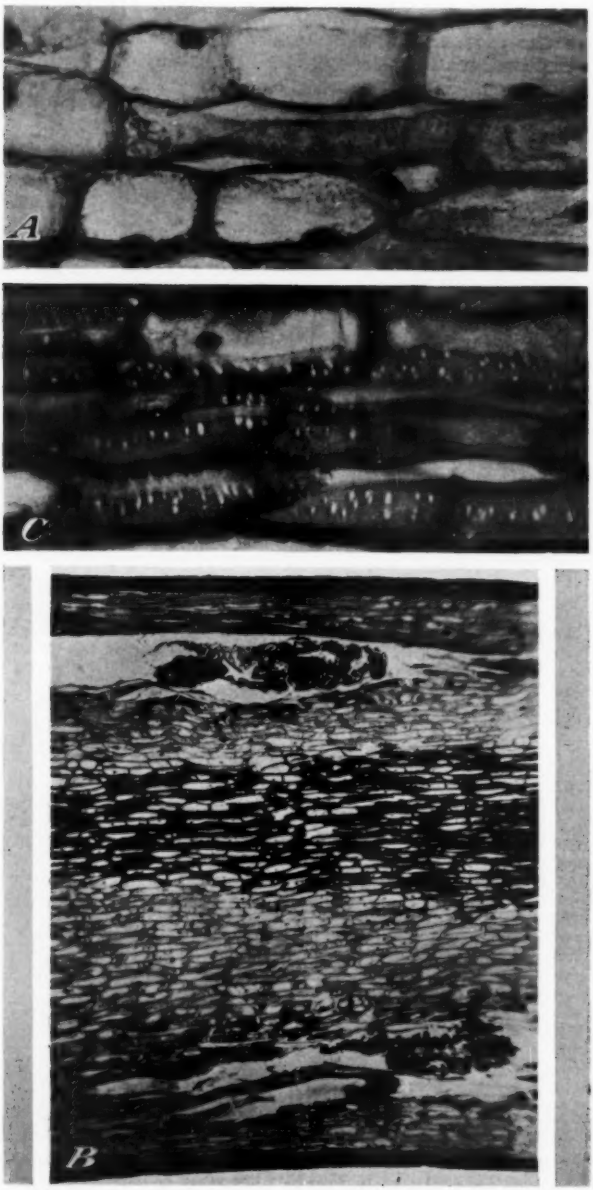

FIGURE 12.—*A*, Part of a longitudinal section through gall tissue around second-instar larvae; note that this tissue is becoming thick-walled, and pits are present in the walls, × 400. *B*, Part of a longitudinal section through gall tissue around third-instar larvae; a part of the tissue between the larvae has become thick-walled and is lignifying, × 25. *C*, a few of the thick-walled cells shown in *B*, highly magnified to show the thick walls and the numerous large pits, × 400.

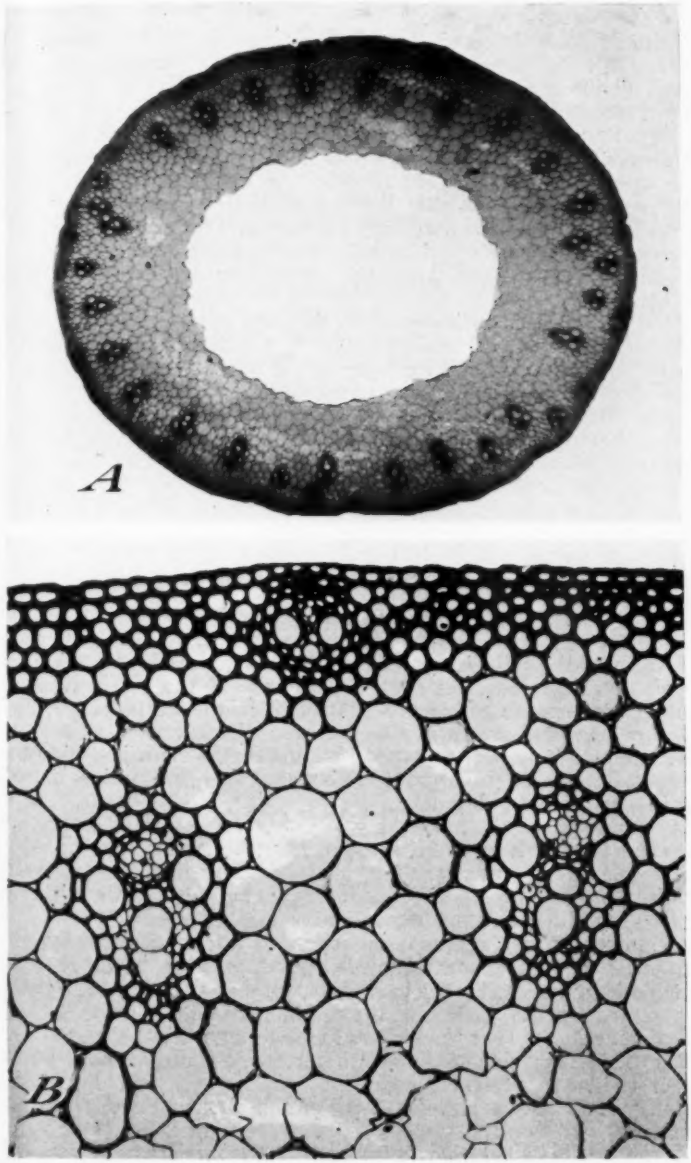

FIGURE 13.—*A*, Transverse section of a normal internode of a wheat culm at the time the jointworm larvae would be full grown, × 20. Note the number, the size, and the regular spacing of the vascular bundles as compared with those in gall tissue illustrated in figures 4 and 11. *B*, A part of the section represented in *A*, × 170.

DISCUSSION

Entomologists have speculated for years as to the reason why *Harmolita tritici* does not occur west of central Missouri and in the Great Plains area. This insect has been known as a serious pest since 1848, and there has been ample opportunity for it to spread into this territory, either by natural means or through the avenues of commerce. The writers believe that this apparently strange phenomenon is explained by the facts presented in this paper. The explanation is very simple. Either the meristematic regions of the wheat culms at the oviposition period are too hard and tough to be acceptable for oviposition in these uninfested areas, or, should oviposition occur, the larvae fail to reach maturity through causes explained in the foregoing discussion.

It is not known at present whether the early hardening of the meristematic region of the internodes in the Kansas wheat plants is due to climatic or varietal differences. If it is varietal, this character could perhaps be transferred to varieties grown in the Eastern and Central States. However, whether this be practical or not, it is known that the insect requires very soft meristematic tissue for oviposition. Therefore, it should be possible through fertilization, or otherwise, to force the growth of the wheat beyond the attractive stage before the adults of the jointworm emerge. Failing in that, it should be practical so to accelerate the growth that oviposition will occur high up the culm. The galls would then be removed by the reaper at harvest and the succeeding infestation thus greatly reduced.

SUMMARY

A study of the gall development in wheat stems following attack by *Harmolita tritici* indicates that the absence of the insect from the Kansas and western Missouri wheat fields is due to the character of the stem in wheat grown in this immense district.

The female locates the vascular bundle in the stem just above an unexposed node by probing for the xylem tubes, and places but one egg in any vascular bundle.

It is difficult for the lancelike ovispositor to pierce the leaf sheath and stem of the wheat plant, consequently the female usually refuses to oviposit in any except the very delicate tissue in the meristematic region just above the node. The larvae do not survive when the egg is placed in older and tougher tissue.

As many as 5 or 6 eggs may be deposited through a single external oviposition puncture, and three such punctures may be made at the same node in practically the same horizontal plane. Eggs are placed only in or near the phloem of the vascular bundle. This is the only gall-making insect that the writers know to have this habit.

The most favorable temperature for oviposition is about 80° F. Under the most favorable conditions the females will deposit the majority of their eggs and die at the end of about 2 days after emergence.

In the Kansas wheat it was found that the meristematic region at the node thought to be susceptible to oviposition was so tough and hard as, doubtless, to be unsuitable. Should oviposition occur in such tissue, the cells would not respond to the stimuli essential for

the development of the normal gall tissue necessary for the nourishment of the larvae of *Harmolita tritici*. This explains why this insect does not occur in the Kansas wheat belt and, perhaps, in certain parts of central Missouri.

Unhatched eggs remain as inert foreign substance embedded in the tissue. This strongly indicates that the female does not inject a stimulating substance into the tissues at the time of oviposition to initiate the production of gall tissue.

Hyperplasia and hypertrophy start at once upon hatching of the eggs, and cell multiplication is by mitosis only. Hyperplasia ceases after the first instar, and is succeeded by hypertrophy. Gall tissue arises partly from the phloem but mainly from the parenchyma, and the cells in the gall area lose their normal polarity. There is no pith cavity in the part of the internode within the gall area, this section of the internode being filled with gall cells. Practically all the vascular tissue is destroyed in this region also, and this greatly interferes with the normal functions of the plant. There are no concentric layers or zones in the gall tissue as is common in the cynipid galls. The entire region lignifies and becomes hard by the time the larvae are full grown.

The gall stimulus is apparently due to byproducts of larval metabolism and, perhaps, to mechanical irritation.

It should be possible to so accelerate the growth of wheat in the early spring, by fertilization or otherwise, that the plants would be unacceptable for oviposition or unsuitable for larval development at the time of attack. Even if this failed, the additional growth caused by such measures would cause the galls to be located so high up on the culms that they would be removed by the reaper at harvest and thus greatly reduce infestation in the following crop.

LITERATURE CITED

(1) Beyerinck, M. W.
1888. ueber das cecidium von nematus capreae auf salix amygdalina. Bot. Ztg. 46: [2]–11, [17]–28, illus.

(2) Cosens, A.
1912. a contribution to the morphology and biology of insect galls. Canad. Inst. Trans. 9 (pt. 3): 297–387, illus.

(3) Docters van Leeuwen-Reynvaan, W., and Docters van Leeuwen-Reynvaan, J.
1907. ueber die anatomie und die entwicklung einiger isosomagallen auf triticum repens und junceum und ueber die biologie der gallformer. Marcellia 6: 68–101, illus.

(4) Hance, R. T.
1933. a new paraffin embedding mixture. Science (n. s.) 77: 353.

(5) Houard, C.
1904. recherches anatomiques sur les galles de tiges: acrocécidies. Ann. Sci. Nat., Bot. (8) 20: [289]–384, illus.

(6) Kinsey, A. C.
1930. the gall wasp genus cynips; a study in the origin of species. 577 pp., illus. [Bloomington, Ind.] (Ind. Univ. Studies 16, nos. 84–86.)

(7) Küster, E.
1911. die gallen der pflanzen, ein lehrbuch für botaniker und entomologen. 437 pp., illus. Leipzig.

(8) Magnus, W.
1914. die entstehung der pflanzengallen verursacht durch hymenopteren. 160 pp., illus. Jena.

(9) Ross, H.
1932. PRAKTIKUM DER GALLENKUNDE (CECIDOLOGIE). ENTSTEHUNG, ENTWICKLUNG, BAU DER DURCH TIERE UND PFLANZEN HERVORGERUFENEN GALLBILDUNGEN SOWIE ÖKOLOGIE DER GALLENERREGER. 312 pp., illus. Berlin.
(10) Smith, E. F.
1917. MECHANISM OF TUMOR GROWTH IN CROWNGALL. Jour. Agr. Research 8: 165–188, illus.
(11) Weidel, F.
1911. BEITRÄGE ZUR ENTWICKLUNGSGESCHICHTE UND VERGLEICHENDEN ANATOMIE DER CYNIPIDENGALLEN DER EICHE. Flora [Jena] 102 (N. F. 2): [279]–334, illus.
(12) Zimmerman, A.
1901. BOTANICAL MICROTECHNIQUE; A HANDBOOK OF METHODS FOR THE PREPARATION, STAINING, AND MICROSCOPICAL INVESTIGATION OF VEGETABLE STRUCTURES. Transl. from German by J. E. Humphrey. 296 pp., illus. New York.
(13) Zirkle, C.
1930. THE USE OF N-BUTYL ALCOHOL IN DEHYDRATING WOODY TISSUE FOR PARAFFIN EMBEDDING. Science (n. s.) 71: 103–104.

U. S. GOVERNMENT PRINTING OFFICE: 1935